International Tungsten Industry Association

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24 November 2008

Dr Ruth M Lunn
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Dear Dr Lunn,

Re: Technical Comments on Draft Background Document for Cobalt-Tungsten Carbide Powders and Hardmetals

On behalf of the International Tungsten Industry Association (ITIA), I enclose technical comments prepared by ARCADIS for ITIA which is registered under Belgian law as a not-for-profit association with scientific purposes in support of the tungsten industry. ITIA's members represent 17 countries and include mining companies, processors/consumers, trading companies and assayers. These include the world's leading manufacturers, importers, and users of hardmetal.

As you will see upon review of those comments, the ARCADIS report identifies very serious concerns with respect to the *Draft Background Document*. ITIA's members therefore seek very careful consideration of the concerns raised in the ARCADIS report. I also encourage you and your colleagues to contact Michael Pardus at ARCADIS directly if there are questions with respect to those comments. Mr Pardus can be reached at:

Tel: +1 412 231 6624 ext 561
Email: michael.pardus@arcadis-us.com.

ITIA members look forward to a continuing dialogue with the NTP and its expert panel in the panel's review of the *Draft Background Document*. As you no doubt realise, its recommendations could have a profound impact on the hardmetal industry. Consequently, we believe it is essential that the panel utilize sound science in carrying out its review and making its recommendations.

Yours sincerely,

Michael Maby
Secretary-General

Enc

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ENVIRONMENT

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Subject:
Draft Background Document for Cobalt-Tungsten Carbide Powders and Hard Metals

Date:
24 November 2008

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B0078028

Dear Dr. Lunn:

On October 10, 2008, the National Toxicology Program (NTP) published in the Federal Register (Volume 73, Number 198) the availability of the *Draft Background Document for Cobalt-Tungsten Carbide Powders and Hard Metals* (sic), which is referred to herein as the Draft Background Document. NTP also requested public comments on the Draft Background Document.

Through the Health, Safety & Environment Committee of the International Tungsten Industry Association (ITIA), ARCADIS has developed comments on the Draft Background Document which are provided for consideration by the NTP and the Cobalt-Tungsten Carbide Powders and Hard Metals Expert Panel (referred to herein as the Expert Panel).

The ITIA is registered under Belgian law as a not-for-profit association with scientific purposes in support of the tungsten industry. ITIA's members represent 17 countries and include mining companies, processors/consumers, trading companies and assayers as well as the world's leading manufacturers, importers, and users of hardmetal.

In the context of this submittal, hardmetal refers to a group of hard and wear resistant refractory composites in which hard tungsten carbide particles are bound together or "cemented" by a ductile binder phase of cobalt unless otherwise noted (Lassner and Schubert, 1999). Tungsten carbide / cobalt hardmetal may also contain auxiliary metal carbides along with other metals in the cobalt binder phase. The term "hard metal" is used synonymously for hardmetal referenced in the Draft Background Document.

SUMMARY OF COMMENTS ON THE DRAFT BACKGROUND DOCUMENT

This report provides a summary of the major technical comments developed for the ITIA regarding the Draft Background Document. It is followed by more detailed technical comments where appropriate. Additional supporting documentation is also provided in the attached annexes.

Based on its review, the following comments are provided by the ITIA regarding the Draft Background Document:

- A detailed causation analysis of the four published hardmetal epidemiological studies identified in the Draft Background Document revealed that they do not represent independent cohorts and fail to establish evidence of carcinogenicity. Lack of a dose-response relationship, inadequate controls for smoking, and significant loss to follow-up are among the substantive deficiencies in those studies.

- The Draft Background Document acknowledges that there have been no studies of the carcinogenicity of cobalt–tungsten carbide powders or hardmetal in experimental animals.

- Hundreds if not thousands of different grades of tungsten carbide hardmetal (i.e., varying concentrations of tungsten carbide and cobalt along with other additives) are now being produced as a result of specific application requirements. Not only is hardmetal chemistry complex, but that chemistry can have significant implications with respect to exposure and physiological effects. Consequently, an assessment of hardmetal toxicity and potential carcinogenicity requires an understanding of the specific grade(s) of hardmetal involved.

- While several cobalt compounds (cobalt sulfate, cobalt chloride, and cobalt naphthenate) and cobalt metal have been reported to cause tumors in experimental animals, ongoing studies of cobalt-containing tungsten alloy using mice have not replicated the tumorgenesis observed in rats. Furthermore, while cobalt sulfate is listed in the *Report on Carcinogens, Eleventh Edition* as *reasonably anticipated to be a human carcinogen* this has no relation to exposures or carcinogenic potential associated with hardmetal.

- IARC concluded that no increased risk of lung cancer was identified for exposure to sintered hardmetal (IARC, 2006, p. 94; NTP, 2008, p. 56). The vast majority of employees in the hardmetal industry work with partially or fully sintered hardmetal. For the small percentage of employees in the U.S. hardmetal industry (estimated to be on the order of 2,000 employees) that work with unsintered hardmetal, their exposures are well controlled through

engineering controls, work practices, and use of personal protective equipment where necessary (Hsu, W., 2004).

- The Center for Disease Control and Prevention (CDC, 2003; Rubin et al., 2007) determined that the incidence of acute lymphocytic leukemia among children in Fallon, Nevada was not related to any environmental exposure, including tungsten. Accordingly, the Fallon investigation cannot provide the NTP or the Expert Panel with any useful information in assessing to the carcinogenicity of hardmetal and should be removed from the report.

For the reasons stated above, it is inappropriate at this time for the NTP or the Expert Panel to develop any recommendation regarding the carcinogenic potential of hardmetal. It is also important to point out that weaknesses noted in the prior epidemiological studies are being addressed by an ongoing epidemiological study being conducted by the University of Pittsburgh and the University of Illinois, Chicago. The current study includes more than twenty facilities located in the U.S., Austria, Germany, Sweden and the UK. This study has been designed to address the weaknesses noted above in the prior studies. It is expected that the study will be completed ca. 2012 and the results will be published in a peer-reviewed scientific journal.

At a minimum, the NTP should very seriously consider deferring its decision with respect to hardmetal until the University of Pittsburgh / University of Illinois, Chicago study is complete. Such a deferral is particularly appropriate here because few employees are exposed to unsintered materials and adequate controls are in place to minimize exposures to such materials.

DETAILED TECHNICAL COMMENTS

The relative dearth of epidemiological data is acknowledged by the NTP in the Draft Background Document. Although not explicitly stated in the Draft Background Document, the available data do not constitute “sufficient evidence of carcinogenicity from human studies” for cobalt-tungsten carbide powders and hardmetals to be classified as a “known human carcinogen.”

The NTP has established three criteria for defining a compound or mixture as *Reasonably Anticipated To Be [A] Human Carcinogen* and, therefore included in the Report on Carcinogens. The following comments are structured to specifically address these criteria.

***<u>Criterion 1</u>: There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded*.**

In the October 25, 2004 issue of the Federal Register (Vol. 69, No. 205), "Cobalt/Tungsten-Carbide Hard Metal Manufacturing" was nominated for review for inclusion in the 12th Report on Carcinogen. The technical basis for the nomination was not fully specified at that time. Nonetheless, the ITIA submitted comments which formed the basis of a detailed Causation Analysis (BBL Sciences, 2005) of the epidemiological studies that were believed to form the basis for the statement that "[r]ecent human cancer studies on the hard metal manufacturing industry show[ed] an association between exposure to hard metals (cobalt tungsten-carbide) and lung cancer."

The Draft Background Document identified four epidemiological studies that were used to evaluate exposure to cobalt–tungsten carbide powders and hardmetal and cancer mortality. These studies are:

(1) a cohort study of Swedish workers at three hardmetal facilities (Hogstedt and Alexandersson, 1990);
(2) a small cohort study of French hardmetal manufacturing workers (Lasfargues et al., 1994);
(3) a multi-plant cohort study of workers at 10 hardmetal producing factories in France, which also included a nested case-control analysis (Moulin et al., 1998); and
(4) a cohort study of the largest factory included in the multi-plant French study (Wild et al., 2000).

As noted several times in the Draft Background Document, these studies were "not mutually independent." Page 53 of the Draft Background Document states "if we are looking for completely independent observations, one should either contemplate [Lasfargues et al., and Wild et al.,] and dismiss the paper by Moulin (1998) or, alternatively, dismiss them and consider only the paper by Moulin et al. (1998)."

These four epidemiological studies are identical to those evaluated in the ITIA Causation Analysis (BBL Sciences, 2005), a copy of which is provided as Annex 1. Since the ITIA Causation Analysis report is part of the public comment record, there is no need to provide another set of comments centered solely on the Causation

Analysis. However, we feel it is important to highlight a few of the more important factors contained in the ITIA Causation Analysis as described below.

1) Exposure not adequately characterized to establish a dose-response.

According to Moulin (Moulin et al., 1998) occupational exposure was assessed using a job exposure matrix. Table 5 of this report indicates that this "semiquantitative" approach was used to evaluate the "lung cancer risk as a function of simultaneous cobalt and tungsten carbide exposure." The following table, from Moulin et al., illustrates the Job Exposure Matrix (JEM) used by these authors in an attempt to characterize exposure to hardmetal dust in the workplace.

JEM Level	# of Samples	Arithmetic Mean ($\mu g/m^3$)	Minimum ($\mu g/m^3$)	Maximum ($\mu g/m^3$)	Geometric Mean ($\mu g/m^3$)
1	0				
2	70	39.37	1	228	20.04
3	59	63.38	0.5	449	18.25
4	98	62.33	2	465	26.41
5	32	87.91	1	515	28.59
6	2	169.00	134	204	165.34
7	3	102.33	34	155	85.36
>7 (8 and 9)	0	No Data			

A number of interesting insights into the exposure characterization of this cohort can be observed from these data:

- An increase in the JEM score was supposed to represent an increase in exposure to hardmetal dust. However, for JEM #2 through JEM #5 the arithmetic means show little variation and the geometric mean exposures are virtually identical.

- The arithmetic mean and geometric mean personal air data for JEM #7 are *lower* than for JEM #6. In addition the maximum air concentrations for JEM #6 and #7 are much *lower* than JEM #3, #4, and #5. This conflicts with the claim that JEM #6 and #7 represent higher exposure categories than JEM #3, #4, and #5.

- JEM #6 and #7 exposure levels are based on only three and two samples, respectively. There are no sample results for JEM #8 and #9.

Based on these and other concerns with the characterization of historical exposure by the French hardmetal workers, as well as the dose-response assessments in the Moulin et al. report (such as by JEM level of "cumulative doses"), and the absence of any quantitative assessment of exposure, other than a dichotomous evaluation of "exposed" versus "unexposed," the data are too uncertain to support a causal association between dose and response.

2) Smoking histories may have resulted in misclassification.

A careful review of the three epidemiological studies clearly indicates that none of the investigation teams adequately addressed the most significant confounding variable in studies of lung cancer in human populations – cigarette smoking. Because the smoking histories were not quantitatively defined in the populations of workers, there is no way of knowing the contribution of this known cause of lung cancer upon the observed results. This is especially troubling given the low number of deaths contained in the studies (especially in Wild et al., 2000) and the relatively low Standardized Mortality Ratios (SMRs) reported for lung cancer. Only a few cases misclassified as to smoking status would have had a dramatic impact on the interpretation of a casual relationship between hardmetal exposure and lung cancer.

Perhaps as evidence of this flaw, the odds ratio (OR) for lung cancer associated with smoking was only 3.38 in the Moulin study. This is significantly lower than the OR typically reported for deaths associated with smoking (Hill, 1965; IARC, 1986). The authors acknowledged that this low risk associated with smoking may be due to misclassification.

Other inadequacies in addressing smoking as a confounding variable include:

- None of the 61 individuals with cancer were actually interviewed to obtain their smoking histories prior to their death (e.g., by a personal or occupational physician), and thus there was a lack of direct information in medical or work histories.
- No medical records were reviewed for independent confirmation of self-reported or proxy-reported smoking histories.
- In 70.5% (43 cancer cases) of the cases, the smoking history was obtained from colleagues.
- In 11.5% (7 cancer cases) of the cases, smoking history was obtained from relatives of the individual with cancer.
- In 18% (11 cancer cases) of the cases, there was **no** information on smoking.

Insufficient evaluation of cigarette smoking as a confounder for lung cancer is also an issue in the Wild et al. report. The following paragraph taken from the Wild report highlights the uncertainty in the assessment of the possible contribution to lung cancer from smoking:

> *"Exposure to smoking was abstracted from the records of the occupational health department; however, the information was sketchy until 1978, when current smoking or non-smoking was recorded but no mention was made of past smoking. Therefore, this information was reassessed by a volunteer group of former workers."*

This indicates that quantitative information on smoking histories was unavailable until 1978, which is almost 30 years after the January 1950 initiation date for exposure considered in the study. Even after the improvement in record keeping, the use of "a volunteer group of former workers" to obtain historical information was likely inadequate, although the authors did not provide any critical analysis of the effectiveness of this approach.

Likewise, Lasfargues et al. relied on plant medical records and interviews of still active workers, and not specific information from the reported cancer cases, to categorize smoking habits into individuals who never smoked, ex-smokers, and

current smokers. Nevertheless, this may have been the best of the studies in extrapolating smoking history because the profile of the proportion of smokers and former smokers to nonsmokers in the hardmetal worker cohort closely matched the proportion in the national sample.

Obtaining information on an individual's smoking history can often be very difficult to accomplish, and thus it is not surprising that the only one or two of the principal studies identified in the Draft Background Document could be used to include hardmetal in the Report on Carcinogens are not unusual in this area. However, given the relatively low SMRs or ORs reported in these studies, the fact that the critical effect observed was purported to be lung cancer, and the limited amount of available epidemiological data, the impact of misclassification on the conclusions regarding the carcinogenicity of hardmetal could be profound.

3) Loss to follow up.

In addition to the problems associated with accurately characterizing the most important confounding variable for lung cancer, an unacceptably high number of workers were lost to follow-up. More than 15% (1,131 workers) of the exposed population was not accounted for in this study. Inclusion of these workers undoubtedly could have changed the reported findings of the study, as they might have increased or decreased the SMRs and ORs. In any event, the missing data could have a significant impact on the SMR / OR calculations, and the authors should not have completed the study until most of the missing workers were found.

These three issues, either alone or in combination, would not necessarily lead to the conclusion that there is a fatal flaw in concluding that hardmetal should be included the Report on Carcinogens on the basis of the one or two. However, they do identify a substantial level of uncertainty associated with these studies. Given the fact that there are so few epidemiological investigations that can be used to properly evaluate human carcinogenicity, and in most cases the purported elevated mortality from lung cancer are so minor, with SMRs often less than 2.0, these studies alone do not provide a sufficient weight of evidence to determine that "there is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible" as provided in Criterion 1.

The University of Pittsburgh, in conjunction with the University of Illinois, Chicago, is currently conducting a government-funded epidemiological study of the hardmetal industry that has been designed to address the weaknesses of the prior studies. The

initial review included more than sixty hardmetal facilities in the U.S. and Europe which identified twenty facilities where appropriate information was available regarding employee work histories, exposures, and medical histories along with country-specific mortality records. The twenty hardmetal facilities that form the basis of his study are located in the U.S., Austria, Germany, Sweden, and the UK.

The cohort of exposed workers in University of Pittsburgh / University of Illinois, Chicago study will be the largest of its kind in the hardmetal industry, exceeding the cohort size of all of the prior epidemiological studies combined. The study design and nested case-control study of lung cancer will enable analytic adjustments for smoking and co-exposures to known or suspected carcinogens. The study design will provide the best available estimates of total and cause-specific mortality risks among workers, overall, and in relation to occupational tungsten carbide / cobalt exposure. A particular strength of this study is its ability to characterize the working history of study members relative to tungsten carbide and cobalt exposures and co-exposures to other known or suspected carcinogens.

The University of Pittsburgh / University of Illinois, Chicago study affords a sufficiently long time period for potential tungsten carbide / cobalt exposure and a sufficiently long observation period to observe cancer outcomes in relation to those exposures. Therefore, this study will be able to detect a true increased risk or to conclude that there is no increased risk if one is not detected. It is expected that the study will be completed ca. 2012 and the results will be published in a peer-reviewed scientific journal.

Criterion 2: There is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset,

This criterion cannot be used in support of the inclusion of hardmetal in the 12th edition of the Report on Carcinogens. As noted on page 63 of the Draft Background Document "[n]o studies of the carcinogenicity of cobalt–tungsten carbide powders or hardmetal in experimental animals were identified."

Criterion 3: There is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance, or mixture belongs to a

well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Studies of tungsten carbide / cobalt (Lison and Lauwerys, 1995; De Boeck et al., 2003) suggest that interaction of metal carbides with cobalt lead to enhanced mutagenicity that is not specific to tungsten carbide. However, the results of such tests have yielded inconsistent results. A 2001 study (Lison et al., 2001) included commercial samples of cobalt metal powder, tungsten carbide, tungsten carbide / cobalt (10%), a tungsten / cobalt substance identified as Co_3W, and a blend of tungsten carbide and Co_3W (20%). A copy of the 2001 study is included as Annex 2. A tungsten carbide / cobalt (6%) sample reconstituted in the laboratory was also tested. *In vitro* test conducted on commercial cobalt and hardmetal samples did not elicit signs of cytocoxicity. Only the "reconstituted" hardmetal sample, which was reportedly used by Lison in earlier studies, exhibited cytotoxicity.

NTP has opined that tungsten carbide / cobalt hardmetal toxicity and genotoxicity appears to be "mediated by both solubilized cobalt ions and through a surface chemistry reaction between cobalt and tungsten carbide that occurs at the particulate level." Corrosion of hardmetal particles through galvanic reactions with concurrent production of reactive oxygen species (ROS) has been proposed as the principal mechanism for release of soluble cobalt ions (Gries and Prakash, 2007 and 2008; De Boek, et al. 2003; Lison. 1996).

As indicated above, factors that affect the corrosion of the hardmetal particles also affect toxicity. As such, the structure and chemistry of the "hardmetal" particles has to be an important consideration in assessing the carcinogenic / mutagenic potential of hardmetal. Synergistic effects have been postulated as an explanation for the observed toxicity effects of tungsten carbide / cobalt hardmetal and tungsten alloys with cobalt (De Boeck et al., 2003; NTP, 2008). However, galvanic corrosion of hardmetal particles is a more likely explanation (Combes et al., 2008). Relevant information regarding tungsten carbide / cobalt hardmetal chemistry and its impact on corrosion characteristics is presented below.

Another important consideration is the scope of materials that are encompassed by the term "hardmetal". This term has been loosely applied in the technical literature cited in the Draft Background Document to include a binary mixture of tungsten

carbide and cobalt. Technically, hardmetal refers to a group of hard and wear resistant refractory composites in which hard metal carbide particles (e.g., tungsten carbide) are bound together or "cemented" by a ductile binder phase which may be cobalt and / or other appropriate metals (e.g., iron, nickel, copper, etc.) (Lassner and Schubert, 1999, p. 321).

Tungsten carbide / cobalt hardmetal may also contain auxiliary metal carbides along with auxiliary metals in the binder phase. Information gathered by the ITIA from its members indicates that the majority of tungsten-containing hardmetal produced in or imported into the U.S. includes such auxiliary metals.

However, even where hardmetal is viewed as comprising only tungsten carbide / cobalt, significant variations in chemistry occur depending upon the content of the chemical constituents. In terms of the constituent amounts, the cobalt content of tungsten carbide / cobalt hardmetal ranges from approximately 3% to 30% (Lassner and Schubert, 1999, p. 321). The cobalt content is controlled during the manufacturing process depending on the end use of the hardmetal product, such as cutting tools and wear resistant parts. Variations in the carbon content (such as a stoichiometric deficit or excess) are controlled through the manufacturing process to achieve a specified carbon to binder ratio that significantly affects the physical and metallurgical properties of the hardmetal.

For tungsten carbide / cobalt hardmetal (with no other additives), the variation in cobalt content, carbon to binder ratio, and powder grain / particle size have significant effects on physical and metallurgical properties, including corrosion resistance. For example, a slight stoichiometric decrease in the carbon content significantly improves corrosion resistance (Greenfield, 2008). Improved corrosion resistance translates into reduced toxicity (Gries and Prakash, 2007 and 2008).

The increase in corrosion resistance of hardmetal with lower carbon content is at least partially due to increased solubility of tungsten in the cobalt binder phase, presumably due to a reduction in the galvanic potential difference between tungsten carbide and the matrix (i.e., cobalt with dissolved tungsten). A similar effect can be produced by increasing the cobalt content, which also decreases the carbon to binder ratio. Since bioavailability of cobalt and other metals from the hardmetal appears to be closely related to corrosion of the metal powder or product (Gries and Prakash, 2007), the increased corrosion resistance translates into reduced bioavailability of cobalt.

A similar situation exists for other tungsten carbide / cobalt hardmetal. Hundreds if not thousands of different grades of tungsten carbide /cobalt hardmetal (i.e., varying concentrations of tungsten carbide and cobalt along with other additives) are produced by varying the chemistry, grain size, and other properties of the hardmetal powder and products. The addition of various metals and metal carbides (titanium, tantalum, niobium, chromium, vanadium, silicon, zirconium, and molybdenum) are used to modify the physical and mechanical properties of the hardmetal products (e.g., improved oxidation resistance). The matrix phase can include additions to or replacement of the cobalt binder depending on the product application. Nickel, iron, and copper as well as cobalt (or mixtures of these and other metals) are used as the binder phase in hardmetal production.

As a result, hardmetal chemistry is highly complex which has significant implications regarding physiological effects. Addition of chromium to tungsten carbide / cobalt hardmetal significantly reduces hardmetal corrosion (Greenfield, 2008). Chromium is soluble in the cobalt matrix and presumably reduces the galvanic potential difference between tungsten carbide and the matrix, which appears to be a significant factor in hardmetal corrosion chemistry (Gries and Prakash, 2008).

Grain size / particle size is also a significant factor for corrosion and toxicity of hardmetal powders (De Boeck et al., 2003). The use of grain size modifiers is common practice in the hardmetal industry to yield specific hardmetal powder blends and products. For some hardmetal applications, vanadium and / or chromium are added as grain growth inhibitors to inhibit particle / grain coarsening (Lassner and Schubert, 1999, p. 341). Smaller grain / particle size typically translates into lower corrosion rates of the agglomerated hardmetal powder and product due to more complete coverage of the tungsten carbide surface by the matrix (i.e., reduced porosity) thereby reducing potential sites for corrosion to occur (Greenfield, 2008; Lassner and Schubert, 1999, p. 341).

Studies conducted by the U.S. military also have demonstrated the effect of chemistry on the corrosion of tungsten-containing alloys. Although tungsten heavy alloys are not the same as hardmetal, there are relevant findings from the military studies which are summarized here. Micrographic analysis of W/Ni/Co and W/Ni/Fe pellets from embedded alloy studies indicates significant matrix corrosion for cobalt but not for iron (Shuster et al., 2008). No corrosion was noted for pellets consisting of tungsten. The authors concluded that corrosion was impeded by formation of passive iron oxides on the surface of the pellets.

Studies by the UK Ministry of Defence (Combes et al., 2008) have identified galvanic corrosion in W/Ni/Co and WC/Co mixtures as the cause of the pitting observed in the embedded alloy pellets. The galvanic corrosion does not appear to be specific to tungsten, but appears to result from direct contact of any metal that is sufficiently separated from cobalt on the galvanic scale.

These galvanic effects can be significantly affected by changes in the matrix chemistry. For example, small additions of iron to the cobalt matrix mitigate corrosion of the alloy. Substitution of copper in the matrix phase results in tungsten corrosion. Based on its studies, the UK Ministry of Defence has concluded that "it is not necessary to assume synergistic effects" to explain the results from the embedded alloy studies.

Assessment of hardmetal toxicity and potential carcinogenicity thus requires a deep understanding of the specific grade(s) of hardmetal involved. In other words, chemistry matters when discussing hardmetal. As previously noted, the proposed mode of action is "mediated by both solubilized cobalt ions and through a surface chemistry reaction between cobalt and tungsten carbide that occurs at the particle level" (NTP, 2008). Galvanic corrosion due to electrochemical potential differences between tungsten carbide and cobalt can give rise to both soluble cobalt ion and ROS (Gries and Prakash ,2007 and 2008).

However, as has been detailed throughout this section, hardmetal chemistry is highly variable and galvanic effects can be dramatically decreased by variations in the chemistry, grain size, porosity, etc. of the hardmetal particles. These considerations apply even when discussing binary tungsten carbide / cobalt hardmetal blends. While the proposed mode of action may be true for some grades of hardmetal, it does not appear to be true for across the spectrum of hardmetal used in the U.S. Additionally, several authors have postulated that the observed effects of hardmetal and tungsten alloys can be explained on the basis of demonstrated galvanic corrosion of some grades of hardmetal and tungsten alloys and at least one author has indicated that these effects are not due to postulated synergistic effects (Combes et al., 2008; Gries and Prakash, 2007 and 2008).

As indicated by the foregoing discussion, hardmetal does not belong to a "well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant

information that the agent acts through mechanisms indicating it would likely cause cancer in humans."

It is also important to differentiate between compounds containing both tungsten carbide and cobalt and those containing only cobalt. Several cobalt compounds (cobalt sulfate, cobalt chloride, and cobalt naphthenate) and cobalt metal have been reported to cause tumors in experimental animals. However, ongoing embedded alloy studies using mice have not replicated the tumorgenesis observed in rats (Roszell, 2008). Thus even though cobalt sulfate is listed in the *Report on Carcinogens, Eleventh Edition* as *reasonably anticipated to be a human carcinogen* it has no relation to exposures associated with hardmetal.

In addition to issues regarding hardmetal chemistry, the form of the hardmetal is also significant. IARC concluded that no increased risk of lung cancer was identified for exposure to sintered hardmetal (IARC, 2006, p. 94; NTP, 2008, p.56). Sintering is a heat treatment process that provides the compacted hardmetal product (e.g., cutting tool, rod, etc.) with the required physical and mechanical properties required for its intended end use. Sintered tungsten carbide /cobalt hardmetal compacts typically approaching 100% of theoretical density.

Sintering is conducted either under a reducing hydrogen atmosphere or in vacuum furnaces. The sintering process allows the hardmetal to compact, modifies grain size, reduces porosity, and changes the chemical composition of the binder matrix (Lassner and Schubert 1999, pp. 348 – 351). Grain size, porosity, and matrix chemistry affect hardmetal corrosion characteristics (including significant reductions in corrosion). Reduction in hardmetal corrosion also reduces the bioavailablilty of cobalt and the concurrent production of ROS.

Given that IARC has not identified an increased risk of lung cancer associated with sintered products, the physical form of the hardmetal, as well as the chemistry, must be thoroughly evaluated in order to reach a scientifically defensible position on "hardmetal" classification by the Expert Panel or the NTP.

The vast majority of employees in the hardmetal industry work with partially or fully sintered hardmetal. For the small percentage of employees in the hardmetal industry that do work with unsintered hardmetal, their exposures are limited through engineering controls, work practices, and use of personal protective equipment where necessary. Indeed, the estimated number of U.S. workers potentially exposed to unsintered hardmetal is only on the order of 2,000 employees. This represents

less than 0.0006% of the total U.S. workforce. Given the controls used throughout the U.S. hardmetal industry, worker to exposure to hardmetal is limited. End user exposures are virtually non-existent (Hsu, W., 2004).

Appendix A. Fallon, Nevada leukemia cluster

It is unclear why a discussion of the purported cancer cluster which occurred between 1997 and 2004 in the city of Fallon, Nevada provides any insight into whether hardmetal is "reasonably anticipated to be a human carcinogen." The residents of Fallon and other areas of the southwestern Nevada have been exposed to naturally-occurring tungsten in groundwater since those areas were settled. In response to concerns raised about the potential cancer cluster, the Center for Disease Control and Prevention has determined that the incidence of acute lymphocytic leukemia in children in Fallon was not related to any environmental exposure, including tungsten (CDC, 2003; Rubin et al., 2007).

Ongoing research activities by the U.S. military, the UK Ministry of Defence, and others demonstrates that tungsten has limited biological implications based on ongoing studies (Pardus et. al, 2009). A copy of this paper is presented in Annex 3. The available studies, some of which are now being published in the peer-reviewed literature indicate that:

- Tungsten is not genotoxic (Prues et al. 2008, Reddy et al. 2007).
- Tungsten does not result in tumors when implanted in rats (Roszell et al. 2008).
- Tungsten is relatively non-toxic (Schell and Pardus, 2009; McInturf et al., 2008; CHPPM, 2008; CHPPM, 2006).

Information on the Fallon investigation does not provide either the NTP or the Expert Panel with any useful information in assessing to the carcinogenicity of hardmetal. Therefore, a discussion of the purported leukemia cluster in Fallon is not warranted, and Appendix A should be removed from the final version of the Background Document.

Conclusions and Closing

For the reasons detailed above, it is premature for the Expert Panel or the NTP to reach a conclusion regarding the classification of hardmetal. While this report has

identified substantive technical reasons that support this conclusion; however, the most compelling rational to defer a decision by the Expert Panel would appear to be the weakness of the published epidemiological studies for the hardmetal industry.

Given the technical weaknesses of the epidemiological studies cited in the Draft Background Document, such studies are technically weak and are insufficient to establish a causal relationship between hardmetal exposure and lung cancer. Indeed the deficiencies in the four published hardmetal studies are acknowledged throughout the Draft Background Document. It is therefore most fortunate that a robust epidemiological study of the hardmetal industry is now underway. That study, which encompasses twenty facilities located in the U.S., Austria, Germany, Sweden, and the UK and is being carried out by the University of Pittsburgh and the University of Illinois, Chicago, has been designed to address the deficiencies in the prior studies. The University of Pittsburgh / University of Illinois, Chicago study is expected to be completed by ca. 2012 and the results will be published in a peer-reviewed scientific journal.

In light of the study that is now underway, the NTP should, at a minimum, very seriously consider deferring its review of hardmetal until the University of Pittsburgh / University of Illinois, Chicago study is complete. Such a deferral is particularly appropriate here because few employees are exposed to unsintered materials and because adequate controls are in place to minimize exposures to such materials.

Sincerely,

ARCADIS

[redacted]

Michael J. Pardus, REM
Vice President / Principal Scientist

Annexes

1 BBL Sciences 2005. Hardmetal Exposure and Lung Cancer: A Causation Analysis.

2 Lison, D., Arras, M., Fubini, B., and Prandi, L. 2001. In vitro tests on cobalt, cobalt compounds and mixtures with tungsten carbide particles. Prepared for Kennametal.

3 Pardus, M., Lemus-Olalde, R. and Hepler, D. 2009 (accepted for publication). Tungsten human toxicity: A compendium of current research on tungsten and tungsten compounds. Journal of Land Contamination. Special Edition on Tungsten.

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Annex 1

BBL Sciences 2005.

Hardmetal Exposure and Lung Cancer: A Causation Analysis

Table of Contents

Acronyms

ACGIH	American Conference of Governmental Industrial Hygienists, Inc.
CSTEE	EU Scientific Committee on Toxicity, Ecotoxicity and the Environment
DFG	Deutsche Forschungsgemeinschaft
IARC	International Agency for Research on Cancer
JEM	job-exposure matrix
MAK	Maximale Arbeitsplatzkonzentrationen
NIOSH	National Institute for Occupational Safety and Health
NTP	National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
SMR	Standard Mortality Ratio
TLV	Threshold Limit Value
UCL	Upper Confidence Limit
USEPA	U.S. Environmental Protection Agency

1. Executive Summary

In July 2004, the Senate Commission of the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) on the Investigation of Health Hazards of Chemical Compounds in the Work Area published its evaluation of the scientific substantiation for the categorization of "hardmetal," the binary mixture of tungsten carbide and cobalt. The purpose of the analysis was an attempt to establish a Maximum Allowable Concentration (or the Maximale Arbeitsplatzkonzentrationen [MAK]) for this mixture. The DFG evaluated the body of scientific information and came to the following conclusion:

> *Hardmetal dusts are rated as category 1 carcinogens since they cause lung cancer in humans. Confirmation is provided by three epidemiological studies with partially overlapping cohorts from France (Lasfargues et al., 1994; Moulin et al., 1998; Wild et al., 2000) and one additional study from Sweden (Hogstedt and Alexandersson, 1990).*

The DFG supported its decision to rate hardmetal as "category 1 carcinogens" in part on an *in vivo* genotoxicity study by De Boeck et al. (2003a) wherein breaks in the DNA strand (using the alkaline comet assay) and micronuclei in type II pneumocytes were observed in rats following intratracheal instillation of hardmetal dust.

The epidemiological studies and genotoxicity cited by the DFG were reviewed, analyzed, and subjected to causation analysis, a broadly accepted and scientifically objective methodology that utilizes a number of criteria in order to establish the existence of a cause-and-effect relationship between chemical exposure and an adverse health outcome, such as cancer. The specific criteria examined in this causation analysis included: 1) consistency of the association; 2) strength of the association; 3) dose- (or exposure-) response relationships; 4) temporality; 5) biological plausibility; 6) confounder analysis; and 7) coherence of the evidence.

The result of this analysis leads to the following conclusions regarding the reviewed epidemiological data:

- The studies provide evidence that the data are inconsistent and inconclusive.
- Confounding factors, especially smoking histories among the examined cohort, were not adequately addressed.
- There was no apparent increase in lung cancer mortality with either increasing exposure (and dose) of hardmetal dust or duration of exposure.
- Although there is a purported increase in mortality from lung cancer, there is no apparent increase in any other lung disease, including Hardmetal Disease.

Application of the causation criteria to the four epidemiological studies cited by the DFG in support of its categorization of hardmetal dust reveal that all four studies were plagued by study design weaknesses (e.g., low number of deaths); uncertainties, particularly in estimating exposure (and, therefore, dose); and an inability to address important confounding variables (e.g., cigarette smoking [see the summary table at the end of this section]). These study design weaknesses are amplified by the fact that three of the studies – Lasfargues et al. (1994), Moulin et al. (1998), and Wild et al. (2000) – are interrelated in that they study the same workforce, although not in its entirety in all studies, and, hence, are not independent investigations. Thus, the weak associations reported by these investigators cannot be used to classify hardmetal dust as "category 1 carcinogens since they cause lung cancer in humans."

Similarly, the DFG seems to place too much reliance on the *in vivo* genotoxicity study by De Boeck et al. (2003a) in support of its classification of hardmetal dust as "category 1 carcinogens." While the DFG report does restate the conclusions reached by De Boeck et al. (2003a), a more rigorous analysis of the data presented by De Boeck and coworkers suggests that their conclusions may be in error. Indeed, a later review of the data by the investigators themselves casts doubt of the finding of a statistically significant increase in DNA migration, as measured in the comet assay, at 12 hours following treatment with a 16.6-milligram tungsten carbide-cobalt mixture per kilogram of body weight (16.6-mg WC-Co/kg). Moreover, the investigators found that only the 16.6-mg WC-Co/kg dosage resulted in a significant increase in micronucleated AT-II cells, while other dosages, even a higher dosage, were not reported in the DFG report. Nor was the possibility that some, or all, of the reported genotoxic effects observed in this study could be due to inflammatory cells resulting from pulmonary toxicity, as noted by De Boeck et al. (2003a) themselves, included in the DFG report. All in all, the *in vivo* data relied upon by the DFG to support its classification of WC-Co as "category 1 carcinogens" are incomplete and controversial, and the DFG's reliance on it seems unwarranted.

Given the weak and limited nature of existing relevant epidemiological and genotoxicity data available at this time, hardmetal should not be rated as "category 1 carcinogens." While data from studies using in vitro system purport a possible biological mechanism for hardmetal causing harm at a cellular level, they, too, are insufficient to support the determination that hardmetal exposure is causally associated with lung cancer in humans.

SUMMARY OF CAUSATION ANALYSIS FOR HARDMETAL

Causation Analysis Criteria	Criteria Met?	Explanation
Consistency of the observed association	No	• Three of the studies investigated the same worker population and did not represent differences in exposure, confounding factors, or other important variables. • Evidence of consistency in response among different populations, engaged in different activities, sharing exposure to a common chemical was not available within the hardmetal epidemiological data.
Strength of the observed association	No	• Due to the limited number of mortalities identified and the consistent lack of statistical significance, these data could not be considered as evidence of a "precise cancer mortality."
Dose- (or exposure-) response relationship	No	• Based on the lack of direct exposure data (or due to errors in the development of the exposure matrix) and the lack of any statistically significant exposure-related effects, this criterion was not met.
Temporal relationship of the observed association	Undetermined	• It was unclear whether the results associated with the population employed for 20 years or more demonstrated that the exposure appropriately preceded the observed effect and that the time interval between the exposure and the observation of the lung cancer is credible.
Biological plausibility	Undetermined	• There is insufficient evidence upon which to make a judgment as to whether tungsten carbide-cobalt mixtures are genotoxic to occupationally exposed humans. • The *in vitro* and *in vivo* data are too limited, conflicting, and insufficient to support the hypothesis that the mixture of cobalt tungsten carbide is capable of transforming normal human pulmonary cells into fatal, highly malignant derivatives.
Elimination of confounders	No	• Smoking history was inadequately accounted for, and smoking is perhaps the most significant confounder for lung cancer. • A high number of workers were lost to follow-up. • Exposure to other International Agency for Research on Cancer (IARC) carcinogens exhibits a statistically significant increase in lung cancer.
Coherence of evidence	No	• The studies failed to lay out a logical and consistent argument supporting a cause-and-effect relationship. • The absence of deaths from fibrosis (including Hardmetal Disease) and pneumoconiosis suggests that high exposure sufficient to cause frank lung toxicity was not present in the studied workers.

2. Introduction

In July 2004, the Senate Commission of the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) on the Investigation of Health Hazards of Chemical Compounds in the Work Area published its evaluation of the scientific substantiation for the categorization of "hardmetal," the binary mixture of tungsten carbide and cobalt. The purpose of the analysis was an attempt to establish a Maximum Allowable Concentration (or the Maximale Arbeitsplatzkonzentration [MAK]) for this mixture. The DFG evaluated the body of scientific information and came to the following conclusion:

> *Hardmetal dusts are rated as category 1 carcinogens since they cause lung cancer in humans. Confirmation is provided by three epidemiological studies with partially overlapping cohorts from France (Lasfargues et al., 1994; Moulin et al., 1998; Wild et al., 2000) and one additional study from Sweden (Hogstedt and Alexandersson, 1990).*

As noted above, four primary studies were the focus of the DFG's evaluation, although the two earliest reports (Hogstedt and Alexandersson, 1990; Lasfargues et al., 1994) have limited statistical power because of the small number of deaths in the identified cohort. The two most significant studies, in terms of number of workers and deaths in the study – the first published by Moulin and co-workers in 1998, and the second by Wild and co-workers in 2000 – are actually investigations on the same group of workers. In fact, this cohort includes workers from the plant in France originally investigated by Lasfargues and co-workers and published in 1994. These four studies (Hogstedt and Alexandersson, Moulin et al., Wild et al., and Lasfargues et al.), which are the basis of the DFG analysis, are summarized and then evaluated using the causation criteria.

In addition, the DFG supported its decision to rate hardmetal dust as "category 1 carcinogens" in part on an *in vivo* genotoxicity study by De Boeck et al. (2003a) wherein breaks in the DNA strand (using the alkaline comet assay) and micronuclei in type II pneumocytes were observed in rats following intratracheal instillation of hardmetal dust. The relevance of these data in providing a weight-of-evidence support for the proposed categorization of hardmetal is discussed under the specific causation criterion of "Biological Plausibility."

3. General Causation Analysis

Causation analysis is an objective scientific approach often attributed to Sir Bradford Hill who developed a set of criteria that were used in the examination of cigarette smoking and lung cancer (Hill, 1965). The "Hill Criteria" as they have come to be known, have been modified over the years by various regulatory agencies (e.g., International Agency for Research on Cancer [USEPA], 1999, 2003), scientific organizations, and individual scientists (e.g., Greim and Deml, 1996) to objectively evaluate epidemiological data. The generally agreed-upon Hill Criteria, also referred to as Causation Criteria, which are used to determine whether an observed association is causal rather than spurious, have been provided recently in the USEPA's *Draft Final Guidelines for Carcinogen Risk Assessment* (USEPA, 2003). The European Commission states in its Technical Guidance Document on Risk Assessment that the contribution of epidemiological studies to causal inference should be evaluated "…using generally accepted causality criteria, such as those of Bradford Hill" (European Commission, 2003; [CSTEE], 2002).

A causation analysis of the currently available epidemiological data for hardmetal powder was performed, and summarized in subsequent sections of this report, using the following seven criteria:

(1) Consistency of the observed association. Consistent findings of the same association in several if not all available independent studies provide the only assurance that the association exists and is not an artifact of the conditions inherent to one particular study. The reproducibility of findings constitutes one of the strongest arguments for causality (USEPA, 2003). If there are discordant results among investigations, possible reasons, such as differences in exposure, confounding factors, and the power of the study, are considered.

(2) Strength of the observed association. The finding of large, precise cancer mortality (e.g., Standard Mortality Ratio > 2) increases confidence that the association is not likely due to chance, bias, or other factors. A modest change in mortality, however, does not necessarily preclude a causal association, but may reflect a lower level of exposure, an agent of lower potency, or a common disease with a high background level.

(3) Dose- (or exposure-) response relationship. A clear dose-response relationship (e.g., increasing effects associated with greater exposure) strongly suggests a cause-and-effect relationship, especially

when such relationships are also observed from duration of exposure (e.g., increasing effects observed following longer exposure times). Because there are many possible reasons that an epidemiologic study may fail to detect an exposure-response relationship (for example, a small range of observed exposure levels or exposure misclassification), the absence of an exposure-response relationship does not exclude a causal relationship.

(4) Temporal relationship of the observed association. This criterion requires that exposure to the suspected causative substance appropriately precede the observed effect and that the time interval between the exposure and the observation of the effect be credible. Because a latent period of up to 20 years or longer is associated with most cancer development, the study should consider whether exposures occurred sufficiently long ago to produce an effect at the time the cancer is assessed. This is among the strongest criteria for an inference of causality.

(5) Biological plausibility. An inference of causality tends to be strengthened by consistency with data from experimental studies or other sources demonstrating plausible biological mechanisms. A lack of mechanistic data, however, is not a reason to reject causality.

(6) Elimination of Confounders. Confounding is the participation of other factors, including exposure to other chemicals, diet, and other socio-economic factors in the development of the observed effect (i.e., cancer). In order to develop a cause-and-effect relationship, the contribution of these other factors must be identified, and adjustments in the analysis must be made. Adjustment for potentially confounding variables can occur either in the study design or in the statistical analysis of the results (USEPA, 2003). Failure to account for confounding variables is not a reason to reject causality.

(7) Coherence of Evidence. The coherence of the evidence essentially deals with the logical consistency and believability of all of the information. An inference of causality may be strengthened by other lines of evidence (e.g., animal bioassays, pharmacokinetic studies) that support a cause-and-effect interpretation of the association. The absence of other lines of evidence is not always a reason to reject causality.

The two major epidemiological investigations (Wild et al., 2000; Moulin et al., 1998), the initial report on French workers exposed to hardmetal dust (Lasfargues et al., 1994), and the study of Swedish hardmetal facilities (Hogstedt and Alexandersson, 1990) were evaluated using these criteria to determine the strength of

evidence for a cause-and-effect relationship between hardmetal dust and lung cancer. It is important to reiterate that, although the publications represent investigations into occupational exposure to hardmetal dust, they include many of the same workers. The Moulin et al. report evaluated 10 facilities, primarily in France, while the Wild et al. study focused on the largest of these facilities and included approximately 40% of the Moulin et al. cohort. Likewise, the Lasfargues et al. (1994) investigation was an early report on one of the plants evaluated by Moulin et al. Thus, these studies cannot be viewed as independent investigations because they do not evaluate discrete populations.

4. Summary of Major Epidemiological Studies

The DFG stated in its scientific substantiation for the categorization of hardmetal as a category 1 carcinogens that the categorization was based on the studies of Lasfargues et al. (1994), Moulin et al. (1998), and Wild et al. (2000) and on one additional study from Sweden (Hogstedt and Alexandersson, 1990). These major studies that formed the technical bases of the DFG categorization are briefly summarized in the following sections.

4.1 "Causes and Death Among Hardmetal Workers" by Hogstedt and Alexandersson (1990)

A cohort of 3,163 male workers involved in the "creation of hardmetal" at three Swedish factories was evaluated. A total of 304 workers died between the years 1951 through 1982. Exposures to cobalt during the various manufacturing processes were estimated; five cobalt exposure categories were developed based on the type of work performed and the duration engaged in that activity.

Information on cobalt air levels in the hardmetal industry was obtained from previous investigations. From these data, retrospective average air cobalt concentrations, by decade, in the respirable zone were assigned to the four exposure categories (the other category was "unexposed to cobalt"). These data indicated that category 1 and category 2 (defined as the "low exposure group") had cobalt air levels ranging from 1 to 5 micrograms per cubic meter ($\mu g/m^3$); category 3 and category 4 (the "high exposure group") had cobalt air concentrations ranging from 10 to 11,000 $\mu g/m^3$. Of the total 3,163 men in the cohort, more than 70% were in the "low exposure group," suggesting this group of workers, in general, was not exposed to substantially elevated levels of cobalt during work activities. In fact, 2,248 (71%) of the workers were exposed to cobalt levels below the Occupational Safety and Health Administration (OSHA) Permissible Exposure Level (100 $\mu g/m^3$), the National Institute for Occupational Safety and Health (NIOSH) REL (50 $\mu g/m^3$), the Swedish exposure limit for an 8-hour period (50 $\mu g/m^3$), and the American Conference of Governmental Industrial Hygienists, Inc. (ACGIH) Threshold Limit Value (TLV) (20 $\mu g/m^3$). Thus, while the study evaluated workers between 1951 and 1982, most were exposed to levels less than current occupational exposure limits.

Among all workers, the causes of death with the highest standard mortality ratios were: leukemia (Standard Mortality Ratio [SMR] = 231), cirrhosis of the liver (SMR = 214), cancer of the pancreas (SMR = 166), and cancer of the prostate (SMR = 162). The SMR for cancer of the lungs and bronchi, based on 17 deaths, was not statistically significantly elevated: SMR = 134 (95% Upper Confidence Limit [UCL] = 77 – 213). When evaluated based on cobalt exposure, the SMR for the low exposure group and high exposure group were

virtually identical (131 and 139, respectively) suggesting that the non-significant increase was not exposure (or dose) related. Of note is that 60% (n = 183) of the 304 deaths in this cohort were in the "low exposure" workers. As noted by the authors: "the excess risk for dying of lung cancer may appear surprisingly small in relation to occupational medicine's previous understanding of 'hardmetal lung'. The explanation is probably to be found partly in the comparably low exposure. . . ."

When lung cancer was evaluated as not only a function of cobalt exposure but also latency (i.e., less than 20 years since first exposure and greater than 20 years since first exposure) similar results were reported. The SMR for the low exposure group with more than 20 years since first exposure (SMR = 202) was similar to the high exposure group with 20 or more years of exposure (SMR = 230). However, there was some evidence of an increase in mortality from lung cancer as a function of time since first exposure. In both the low and high exposure groups, the SMR increased with time since initial exposure. The lung cancer SMRs for the low exposure group increased from 113 (1 to 19 years) to 202 (for 20 or more years); for the high exposure group, the SMR increased from 77 to 230.

The cause of death as a function of cobalt exposure was evaluated in several different ways. The only assessment that resulted in a statistically significant increase in lung or bronchial cancer was among all exposed workers (categories 1 through 4) with at least 10 years of exposure and more than 20 years since their first exposure. There was nearly a threefold increase over the expected number of deaths from lung cancer (SMR = 278; 95% UCL: 111 – 572). The authors concluded that "long-term cobalt exposure appears to increase the risk for lung cancer."

4.2 "Lung Cancer Mortality in a French Cohort of Hard-Metal Workers" by Lasfargues et al. (1994)

Lasfargues and co-workers investigated one hardmetal production plant located in central France that started operation in 1956. At the time of the study, the facility employed only 380 workers. The total cohort included only 709 workers, with only 75 reported deaths; 26 of these deaths were attributed to "all malignant neoplasms," and 10 were attributed to malignancies of the "trachea, bronchus, and lung."

The plant had two production workshops. The first workshop, operating since 1956, represented the work area with the highest potential exposure to hardmetal dust. "The exposure to hard-metal dust in the second workshop is lower due to preventive measures taken since its opening in 1974." Although the cohort definition included

all men employed for at least one year, starting on January 1, 1956 (i.e., in the highest exposed workshop), "unfortunately, data concerning individual job histories before 1970 were often missing. Therefore, a possible dose-response relationship for mortality could be assessed only indirectly."

Accordingly, a crude estimate of hardmetal dust exposures was developed (Degrees 1 through 4). These "degrees of exposure" were based primarily on job category and use of some limited quantitative exposure information that was available in the form of cobalt dust concentrations and cobalturia. However, these data were collected only once, during 1983. Thus, these "degrees of exposure" were more qualitative metrics than actual measures of hardmetal exposure. "Data concerning complete individual job histories were not available because precise dates and duration of employment at some exposed workplaces were unknown for many workers, or that time since first exposure and duration of exposure could not be determined exactly…. Classification into four degrees of exposure could be established for the knowledge of dust pollution at the workplaces, but it was not possible to calculate cumulative exposure doses for total dust and cobalt." These concessions highlight the limited utility of this study in establishing a causal relationship between exposure to hardmetal dust and lung cancer in workers.

Similarly, the analysis of the cancer mortality incidence does little to support a cause-and-effect relationship. Although a significant increase in lung cancer mortality was observed among the entire cohort (SMR = 2.13; 95% define [CI] = 1.02 – 3.93), SMRs did not increase according to duration of employment and to time since first employment in the medium and high exposure groups. In other words, no dose-response relationship could be established.

4.3 "Lung Cancer Risk in Hardmetal Workers" by Moulin et al. (1998)

The study by Moulin and co-workers is a nested case-control analysis conducted on workers employed for at least 3 months at 10 hardmetal facilities, from the time the facilities opened until December 31, 1991. Most of the facilities were located in eastern France. The cohort consisted of 7,459 workers (5,777 males). Cases were workers who died of lung cancer; three controls were assigned for each case. Workers were followed from 1968 to 1991, and a total of 684 deaths (63 from lung cancer) were included in the analysis. Occupational exposure to hardmetal dust was assessed using a "Job Exposure Matrix" (JEM) that provided semi-quantitative scores for 320 job periods based on 744 air samples. The authors reported that the death rate for lung cancer was significantly increased (SMR = 1.30; 95% CI = 1.00 –1.66). As noted by Monson (1980) if a confidence interval contains 1.0, a true mortality rate of 1.0 is possible, and suggests that the data in the study are too few to

enable an unequivocal conclusion of a causal association. Deaths from lung cancer in exposed workers, expressed as odds ratios (ORs), increased with cumulative exposure – based on the JEM. For the lowest quartile, the OR equaled unity (i.e., 1.00), and, with increasing exposure quartile, the ORs were 2.64, 2.59, and 4.13, respectively. However, only the highest two quartiles were statistically significantly different from the lowest exposed group. There was no apparent association between death from lung cancer and duration of exposure.

When smoking was included as a co-variant in the analysis, a slight but non-significant increase in the odds ratio was detected. For nonmalignant disease, "this study failed to confirm the known pulmonary toxicity of hardmetals."

The authors concluded that this study supported the hypothesis that workers who manufacture hardmetals have an increased mortality from lung cancer due to simultaneous exposure to cobalt and tungsten carbide. However, the cohort study exhibited only a 30% increase in deaths from lung cancer, and this increase was "of borderline statistical significance."

4.4 "Lung Cancer Mortality in a Site Producing Hardmetals" by Wild et al. (2000)

The study conducted by Wild et al. evaluated the mortality among workers in the largest of the production sites included in the previous report by Moulin et al. (1998), a facility that had been in operation since the late 1940s. The original cohort in this study consisted of 3,398 subjects, but was reduced to 2,860 subjects (2,216 men and 644 women) after the application of certain censoring criteria (e.g., incomplete working histories). The study population comprised all subjects who had worked at the site for at least 3 months. To quantify exposure to hardmetal dust, the JEM previously described in Moulin et al. (1998) and duration in the workshops in which the subjects worked were used in the statistical analysis. The total number of deaths from all causes by January 1, 1968 was 399; 47 were attributed to lung cancer. The significant findings of this study include:

- A weak association was found between exposure to hardmetal dust and smoking (i.e., individuals exposed to hardmetal dust were more likely to be smokers).
- For the entire cohort, without regard to job classification, a significant increase in mortality from lung cancer was observed in men (SMR = 1.70; 95% CI 1.26 – 2.26) but not in women.

- Among workers involved in hardmetal production, without the distinction of sintering, a statistical increase in mortality from lung cancer was observed (SMR of 1.93; 95% CI 1.05 – 3.23).

- Consistently high SMRs were found among workers ever involved in (SMR = 2.42; 95% CI 1.10 – 4.59) and only employed in (SMR = 2.91; 95% CI 1.06 – 6.34) hardmetal production steps before sintering.

- Exposures to chemicals considered by IARC as carcinogens resulted in a significant increase in mortality due to lung cancer (SMR = 2.56; 95% CI 1.28 – 4.59).

- Workers engaged in maintenance activities (only or ever), with non-quantifiable exposures to hardmetal dust, had consistently elevated SMRs for lung cancer. "These increased risks are difficult to interpret as several possible carcinogenic exposures had been coded by the experts who developed the JEM."

The authors concluded that an excess mortality from lung cancer was found among workers producing hardmetals and among maintenance workers, which cannot be attributed to smoking alone. The excess appears mostly in subjects exposed to unsintered hardmetal dust.

5. Causation Analysis of Major Hardmetal Studies

The studies of French hardmetal facilities (Lasfargues et al., 1994; Moulin et al., 1998; Wild et al., 2000) and the study of three Swedish facilities (Hogstedt and Alexandersson, 1990) were subjected to a formal causation analysis using the seven causation criteria previously described. Although each study was independently evaluated in this manner, it is important to remember that the studies actually addressed many of the same individuals. That is, the individuals comprising the Wild cohort were also a significant proportion of the Moulin et al. cohort. Therefore, Wild et al. represents, to some degree, a re-evaluation or update of the Moulin et al. report, and is not a distinctive, diverse cohort. Similarly, the plant examined in the Lasfargues et al. (1994) report was one of the 10 plants investigated by Moulin et al.. Thus, it would not be unexpected that there could be similar findings between the three reports. Acknowledging this limitation in the available data is an important consideration as the studies are evaluated in a formal causation analysis.

Consistency of the observed association

Consistent findings of the same association in several if not all available studies provides the only assurance that the association exists and is not an artifact of the conditions inherent to one particular study. Unfortunately, as previously mentioned, the Moulin et al. (1998) study, along with Wild et al. (2000) study, provide the only investigations with study designs generating the statistical power to detect a true increase in cancer mortality. Because these two studies investigated the same worker population and, thus, do not represent differences in exposure, confounding factors, or other important variables, there are too few additional available studies to satisfy the criterion of consistency.

Other studies on hardmetal workers have been published in the scientific literature, and their results would appear to support the findings reported in the study by Moulin et al. However, these reports are of limited utility in demonstrating consistency in the observed association because of the limited power of the studies. For example, the report by Hogstedt and Alexandersson (1990) on hardmetal workers in Swedish factories reported a similar (30%) increase in lung cancer among its workers (SMR = 134), but it dealt with only 17 lung cancer deaths. The only other mortality study of hardmetal workers, Lasfargues et al. (1994), was a preliminary investigation of the facility included in both the Moulin et al. and Wild et al. reports and included only 10 “trachea, bronchus, and lung” cancers. These investigators stated: “because of the small numbers involved, no firm conclusion should be drawn from this study.”

Because three of the four studies on hardmetal workers relied upon by the DFG, including the two largest investigations, consider the same worker population, there is no way of demonstrating that the weak finding of an increased risk for mortality from lung cancer is not an artifact of the conditions inherent to one particular study. Typically, chemicals designated as "known human carcinogens" have been investigated in many different cohorts, often from several different countries, and, in all of these studies, a consistent finding was reported. For example, benzene exposure and its link to cancer has been investigated in workers not only from different facilities, but also engaged in different industries. Evidence of cancer has been obtained from studies on workers in the rubber, petrochemical, and pliofilm production industries (Budinsky et al., 1999). It is this consistency in response among different populations, engaged in different activities, sharing exposure to a common chemical, that provides the evidence in support of a cause-and-effect relationship. This type of evidence is not available within the hardmetal epidemiological data; therefore, the criterion of consistency has not been satisfied.

Furthermore, the criterion of consistency deals with uniformity in the nature of the response, including the type of cancers reported in the study. In both the Moulin et al. and the Wild et al. studies, "cases" were defined simply as "the cohort workers who had died of lung cancer" while the report did not specify as to the type of lung cancer (cell type and specific tissue involved). Thus, it appears any and all cancers of the lung were grouped together, although specificity of tumor type in the lung is known to be related to the causative agent. For example, cigarette smoking has been shown to be commonly associated to squamous-cell and small-cell carcinomas and not to adenocarcinomas (IARC, 1986). Likewise, vinyl chloride is specifically associated with angiosarcoma (USEPA, 2003). Based on the generality of the grouping in the Moulin et al. and Wild et al. studies, one cannot determine if all of the "lung cancers" observed either within a particular study, or between the two reviewed studies, demonstrated a consistent biological response.

An interesting finding reported by Lasfargues et al. was that the SMRs for all causes, all cancers, accidents and violence, and deaths from suicide all increased according to degree of hardmetal exposure. In fact, the highest SMR reported in the study (6.03) was for suicide among the high exposure group. These results further highlight the lack of consistency in the purported cause-and-effect relationship between hardmetal exposure and lung cancer.

Strength of the observed association

In the Moulin et al. cohort, there was only a 30% increase in deaths from lung cancer. Not only was this increase "borderline" relative to statistical significance, but it also hardly qualifies as the "large, precise cancer mortality" necessary to satisfy this criterion. Also, a standard mortality ratio of less than 2.0 has been viewed by many scientists as insufficient to demonstrate that a particular illness or condition was more likely than not caused by the toxic agent (Taubes, 1995; Norris et al., 2004).

The entire male cohort in the study conducted by Wild and co-workers exhibited an increase in lung cancer, although, like the Moulin et al. study, there was less than a twofold increase (SMR = 1.70; 95% CI 1.24 – 2.26). When the cohort was further segregated into subcategories of exposure (e.g., employed in production only before sintering), the SMRs increased slightly, although the number of deaths attributed to the particular category was always less than 10, and none of the elevated SMRs reached statistical significance, with the exception of co-exposure to "IARC carcinogens."

Lasfargues et al. reported a statistically significant increase in lung cancer, with SMRs exceeding 2.0. However, this finding is based on only 10 observed cases. Interestingly, as the number of workers included in the cohort and the number of deaths attributable to lung cancer increased, the SMRs decreased. This trend toward unity cast doubt on the strength of the reported association.

Lasfargues et al., 1994	709	2.13	1.02-3.93
Wild et al., 2000	2216	1.70	1.24-2.26
Moulin et al., 1998	5777	1.29	0.99-1.66

In the study of Swedish workers, the SMR for cancer of the lungs and bronchi, based on 17 deaths, was not statistically significantly elevated (SMR = 1.34). Even when evaluated based on cobalt exposure, the SMRs for the low exposure group and high exposure group were virtually identical (1.31 and 1.39, respectively) and represented only an approximate 30% increase. Interestingly, including this study in the above table would further illustrate the trend toward unity. The number of male workers in this study was 3,163, and the non-significant increased SMR was 1.34, which places this report between the Wild et al. and Moulin et al. studies.

The questionable significance of SMRs of less than 2.0, as reported for hardmetal exposure and lung cancer, was addressed in an article by Taubes (1995). When one considers the uncertainties associated with accurately characterizing exposures and biological plausibility, and addressing confounding and sampling bias, many epidemiologists insist that no single epidemiological study is pervasive unless there is a three- to fourfold risk increase. Since the Wild et al. and Lasfargues et al. cohorts are only subsets of the larger Moulin et al. cohort, some of the biases, measurement errors, and confounding factors are identical and, to some degree, cannot be viewed as independent studies. Because none of the four studies used by the DFG reported a three- to fourfold increase in risk, these investigations did not satisfy the criterion of strength of association.

Given the limited number of mortalities identified in these categories, and the consistent lack of statistical significance, these data could not be considered as evidence of a "precise cancer mortality"; therefore, the criterion of "strength of the association" has not been satisfied.

Dose- (or exposure-) response relationship

In all four of the studies, problems existed with the efforts to quantitatively define hardmetal exposures. In the Hogstedt and Alexandersson report, retrospective average air cobalt concentrations, by decade, in the respirable zone were assigned to the four exposure groups (the other category was "unexposed to cobalt"). These data indicated that categories 1 and 2 (defined as the "low exposure group") had cobalt air levels ranging from 1 to 5 $\mu g/m^3$; categories 3 and 4 (the "high exposure group") had cobalt air concentrations ranging from 10 to 11,000 $\mu g/m^3$. The specific information on this air sampling was provided in other studies and not reviewed. Of the total 3,163 men in the cohort, more than 70% were in the "low exposure group," suggesting this group of workers, in general, were not exposed to substantially elevated levels of cobalt during work activities. In fact, 2,248 (71%) of the workers were exposed to levels below the current OSHA PEL (100 $\mu g/m^3$); the NIOSH REL (50 $\mu g/m^3$); the ACGIH TLV (20 $\mu g/m^3$); and, according to the authors, the Swedish exposure limit for an 8-hour period (50 $\mu g/m^3$). It is important to note that exposure was characterized solely as "cobalt" and not hardmetal (i.e., cobalt tungsten carbide).

In the Moulin et al. and Wild et al. reports, because there were no measurements that could be used to develop an estimate of a dose (e.g., biomarker data such as blood or urine) or even actual exposure data (e.g., personal air monitoring data), a surrogate for exposure was developed by the investigators. A JEM was developed by a committee of nine experts and assigned semiquantitative estimates of exposure to cobalt and tungsten carbide

based on 320 job periods. In addition, atmospheric concentrations of cobalt were available from previous studies and were used in an attempt to validate the matrix.

Use of the empirical data illustrate that the JEM was not successful in discriminating exposure conditions and, therefore, cannot be used to establish a dose-response effect. The following table is a reproduction of Table 1 from the Moulin et al. report.

JEM LEVEL COMPARED TO MEASURED COBALT AIR CONCENTRATIONS

1	0				
2	70	39.37	1	228	20.04
3	59	63.38	0.5	449	18.25
4	98	62.33	2	465	26.41
5	32	87.91	1	515	28.59
6	**2**	169.00	134	204	165.34
7	**3**	102.33	34	155	85.36
>7 (8 and 9)	**0**	**No Data**			

A number of interesting insights into the exposure characterization of this cohort can be observed from these data:

- Although an increase in the JEM score was supposed to represent an increase in exposure, there is little difference in exposures across JEM #2 through #5 for the arithmetic mean and the geometric mean. As noted by the USEPA (1989, 1992), an average concentration is the most appropriate matrix for evaluating cancer risks for most compounds because cancer is a response to a chronic exposure, and the average concentration is most representative of the concentration experienced over time.
- The arithmetic mean and geometric mean personal air data for JEM #7 are *lower* than JEM #6; these values are based on only three and two samples, respectively.
- No analyses were performed on these data to establish that they were statistically different from each other.
- For the maximum air concentrations, it can be seen that JEM #6 and JEM #7 are much *lower* than JEM #3, #4, and #5. This is in conflict with the claim that JEM #6 and JEM #7 represent higher exposure categories than JEM #3, JEM #4, and JEM #5.

- For JEM exposure categories #8 and #9 (the two highest categories) there were no exposure data, so it is unclear how this information assists in the exposure assessment.

Another interesting, and potentially disconcerting, issue involving the exposure estimate is that the earliest exposure data were from 1971, whereas the first two factories evaluated in this study opened in 1942 and 1945. Hence, earlier exposure levels for many of the cancer cases were not included; more than 93% of the cancer cases were hired before 1970.

Perhaps reflective of the lack of an adequate exposure characterization of the worker population, the authors were unable to demonstrate an exposure-related effect. In fact, the only "exposure levels" with a statistically significant elevated lung cancer mortality were observed in the lowest exposure grouping, levels 2 and 3 (OR = 3.37; 95% CI 1.19 – 9.56). The ORs for lung cancer in all other groupings based on JEM levels (i.e., 4 and 5, and 6 through 9) were not significantly elevated, and the exposure-related trend analysis also was not statistically significant. Thus, based on the errors in the development of the exposure matrix, and the lack of any statistically significant exposure-related effects (which may be a function of the flawed JEM) the results do not satisfy the criterion of demonstrating a dose-response relationship.

In the Lasfargues et al. report, exposure to hardmetal dust was even less quantitative then in the two later studies. Workers were assigned to four "degrees" of exposure (nonexposed, low, medium, and high), and these categories were based largely on job description and location. Even this approach encountered difficulties because individual job histories for many of the workers (those employed prior to 1970) were often missing. Also, nonclerical workers employed prior to 1974 without job histories were arbitrarily assigned to degree 3, the medium, or second highest, exposure category.

The authors acknowledged that "data concerning complete individual job histories were not available because precise dates and duration of employment at some exposed workplaces were unknown for many workers, or that time since first exposure and duration of exposure could not be determined exactly…. Classification into four degrees of exposure could be established for the knowledge of dust pollution at the workplaces, but it was not possible to calculate cumulative exposure doses for total dust and cobalt." These uncertainties made the establishment of a dose-response relationship for mortality from lung cancer impossible. Perhaps reflective of these weaknesses in the exposure estimates, the SMRs for all causes, all cancers, accidents and violence, and

deaths from suicide all increased as a function of "degree of exposure." This apparent "trend" for deaths from causes other than lung cancer calls into question the method for determining exposure to hardmetal dust.

Temporal relationship of the observed association

Cancer from occupational exposures typically has a latency period of approximately 20 years (USEPA, 2003). In the event that a chemical causes cancer, longer intervals between first exposure (start of employment) result in a larger number of exposure-related cancer cases. This is because chemically induced cancer takes time to develop and be observed (diagnosed and/or death). This is particularly true for lung cancer. Therefore, we should observe an increase in the SMRs as time since first exposure increases above 20 years.

The only assessment of Swedish workers (Hogstedt and Alexandersson) that resulted in a statistically significant increase in lung or bronchial cancer was when they combined all exposed workers (categories 1 through 4) with at least 10 years of exposure and more than 20 years since their first exposure. There was nearly a threefold increase over the expected number of deaths (SMR = 278; 95% UCL: 111-572). Thus, under these statistical constraints, a temporal relationship was observed. However, the finding was based on only seven deaths from lung or bronchial cancer (2.5 were expected), which represented only 2% of the mortalities reported among the cohort.

In the Moulin et al. cohort, 33% of the lung cancer deaths (20 of 61) occurred before the individuals had reached 20 years since the start of employment (and, presumably, the initiation of exposure to hardmetal). For this sector of the cohort, temporality seems not to be satisfied. The SMRs for lung cancer mortality in groups employed for 20 to 29 years and greater than 30 years were elevated (1.42 and 1.25, respectively), although the authors did not provide the data to determine whether this moderate elevation was statistically significant. Also, there was no increase in the SMR with increasing time since first exposure, and, as shown in the table below, the SMRs remain relatively constant over time. As such, it is unclear whether the results associated with the population employed for 20 years or more demonstrate that the exposure appropriately preceded the observed effect, and that the time interval between the exposure and the observation of the lung cancer is credible. Thus, it appears that the criterion of temporality was not satisfied in this study.

Latency (time since first employment)	SMR for Lung Cancer
0-9	0.74
10-19	1.33
20-29	1.42
> 30	1.25

Wild et al., in the nested case control study of the Moulin et al. cohort, did not evaluate a change in SMR as a function of latency.

In the study by Lasfargues and co-workers, the SMR for lung cancer was only statistically significant in those individuals with time since first employment of 10 to 19 years, while, the SMR was not significantly elevated for those with 20 or more years since first employed, and was lower than the 10- to 19-year group (3.65 and 2.17, respectively). As noted by the authors, their study failed to demonstrate temporality because the SMRs "did not increase according to duration of employment and to time since first employed in the medium- and high-exposure groups.

Biological plausibility

The likelihood of a causal association between exposure to a substance and an adverse health outcome is strengthened if there exists a biologically plausible mechanism, firmly grounded in science, to explain how the substance leads to the initiation and/or progression of disease. In the case of colbalt-tungsten carbide mixtures, genotoxicity has been advanced and investigated as a possible mechanism by which hardmetals may cause cancer in humans. However, given the inconsistent results observed between *in vitro* and *in vivo* experimental studies, together with the lack of a genotoxic effect in workers exposed occupationally to cobalt or colbalt-tungsten carbide powders, it is not clear whether colbalt-tungsten carbide mixtures are, in fact, genotoxic to humans at levels encountered in the workplace.

Genotoxicity of colbalt-tungsten carbide mixtures has been assessed *in vitro* in human lymphocytes using alkaline elution, comet, and micronucleus assays (Anard et al., 1997; Van Goethem et al., 1997; De Boeck et al., 1998; De Boeck et al., 2003a). Under the conditions employed, colbalt-tungsten carbide mixtures were found to be clastogenic. However, the result of these studies must be viewed cautiously, for several reasons. First, the cells used in these studies were collected from a very small population of humans. For example, in the study by

De Boeck et al. (1998) lymphocytes were collected from three healthy, non-smoking females who were less than 30 years of age. Van Goethem et al. collected cells from one human (sex not specified) who was less than 30 years of age, while De Boeck et al. (2000) collected cells from only two individuals, one male and one female, who were both reportedly less than 28 years of age. Anard et al. (1997) failed to specify from how many individuals cells were collected. Given the few individuals studied, the results of these studies must be viewed as preliminary.

Secondly, in most of these studies, there was a substantial inter-experimental and inter-donor variability, casting some doubt as to the interpretation of the results. Indeed, De Boeck et al. (2003a) noted that, because substantial inter-experimental and inter-donor variation was observed in their study, "the current data need to be considered as preliminary." And, finally, the cells used in these experiments were obtained from peripheral blood and not from the lung, the latter of which is suspected as the target of carcinogenicity of colbalt-tungsten carbide mixtures. Whether or not cells from the lungs, such as type II pneumocytes, would respond similarly has not been investigated. Thus, these studies do not provide evidence of genotoxicity in cells of the lungs.

These studies have been used to advance a biologically plausible mechanism for genotoxicity mediated by the generation of reactive oxygen species that randomly attach and fragment DNA, as detected in these *in vitro* tests. However, for more than a decade, scientists have realized that a positive outcome in short-term *in vitro* tests does not demonstrate a biologically plausible link to cancer in humans.

Several years ago, scientists from the National Institute of Environmental Health Sciences engaged in an extensive review of the *in vivo* and *in vitro* data available on 77 chemicals studied as part of the National Cancer Institute and the National Toxicology Program (Tennant et al., 1990). The purpose of this assessment was to provide "a thorough evaluation of the ability of these [*in vitro*] tests to predict rodent carcinogenicity." [Note that these authors are not even addressing the issue of extrapolating animal studies to human response.] The conclusions reached by the panel of researchers clearly highlight the uncertainty associated with the information obtained from these types of studies and that concludes a mechanistic link to cancer. While arguably consistent with the thinking in the 1970s, this direct extrapolation is no longer reliable based on today's body of scientific knowledge. These scientists stated:

> *The standard against which the performance of STTs [short-term tests] is measured has changed dramatically in the past decade. The high level of concordance [between* in vitro *results and carcinogenicity] published in the early 1970s were accurate at the time. Nearly all known carcinogens tested were genotoxic…*

> *For more than a decade, the dominant paradigm motivating the use of STTs to predict chemical carcinogenesis has been that carcinogens are mutagens and, by implication, that mutagens are carcinogens. On the basis of the results presented here, it is clear that strong qualifications to these associations are needed. No single* in vitro *STT adequately anticipates the diverse mechanisms of carcinogenesis; and more important, the advantage of a battery of* in vitro *STTs is not supported by results of the present study.*
>
> *It is clear that even with a battery of assays, not all rodent carcinogens are* in vitro *mutagens nor are all* in vitro *mutagens rodent carcinogens. If current* in vitro *STTs are expected to replace long-term rodent studies for the identification of chemical carcinogens, then that expectation should be abandoned*. [emphasis added]

Thus, this single effect (i.e., direct damage to cellular DNA) alone does not explain the development of cancer in humans. As recently described in great detail by Hanahan and Weinberg (2000), tumorgenesis in humans is a multistep process with several rate-limiting, stochastic events that are critical to the progressive transformation of a normal human cell into highly malignant derivatives. As a caution to over-interpreting the results of *in vitro* studies, the authors noted:

> *By simplifying the nature of cancer — portraying it as a cell-autonomous process intrinsic to the cancer cell — these experimental models have turned their back on a central biological reality of tumor formation* in vivo*: cancer development depends upon changes in the heterotypic interactions between incipient tumor cells and their normal neighbors.*

The DFG's classification of hardmetal dust as category 1 carcinogens was "supported," in part, on the *in vivo* study by De Boeck and coworkers (2003a). The DFG noted that this study found "genotoxic effects occurring in rats after intratracheal instillation of hardmetal dust in the form of breaks in the DNA strand and micronuclei in type II pneumocytes."

However, information presented in the study by De Boeck et al. (2003a), and elsewhere, calls into question the conclusion that hardmetal dust is mutagenic toward rat type II pneumocytes. In regard to the finding of a statistically significant increase in DNA migration in AT-II cells at 12 hours after intratracheal instillation of 16.6 mg WC-Co/kg discussed above, it should be noted that, in a separate review of the genotoxicity and carcinogenicity of cobalt by De Boeck et al. (2003b), the alkaline comet assay *in vivo* genotoxicity results reported in De Boeck et al. (2003a) for WC-Co in rat type II pneumocytes at a dosage of 16.6 mg WC-Co/kg were discussed and reported as **negative**. This is not consistent with the reported statistically significant increase in DNA migration observed at 12 hours after intratracheal instillation with 16.6 mg WC-Co/kg in comparison with vehicle controls as described above. One possible reason for this inconsistency is that, upon reflection, the authors could not report a positive result for the AT-II cells due to the fact that the positive

control (bleomycin) did not cause a statistically significant increase in DNA damage as detected by the comet assay, suggesting that there could have been a problem with the assay. Another possible explanation might be that this statistically significant positive result for the 16.6-mg WC-Co/kg dosage was seen only at 12 hours post instillation, and the effect dissipated and was no longer present at later time points, again calling into question the relevance of this finding. Either way, the fact that the authors themselves first report a positive response for the AT-II cells exposed to 16.6 mg WC-Co/kg in the comet assay, but then later indicate otherwise, calls into question the validity of comet assay results reported by De Boeck et al. (2003a). Thus, the DFG's statement that "the DNA tail length was significant after 12 hours" is not scientifically reliable, weakening any reliance on this aspect of the study by De Boeck et al. (2003a).

In regard to the findings of a statistically significant increase in micronucleated AT-II cells 72 hours after intratracheal instillation, it is of importance that none of the other dosages used in this study, including the higher dosage of 49.8 mg WC-Co/kg, resulted in such a statistically significant increase. That is, the statistically significant increase in micronucleated AT-II cell was observed only at 16.6 mg WC-Co/kg; it was not observed at dosages of 1.8, 5.5, or 49.8 mg WC-Co/kg. While one might argue that the threshold for such a response was 16.6 mg WC-Co/kg, the fact that the 49.8 mg WC-Co/kg dosage did not cause such an effect suggests that the findings observed at 16.6 mg WC-Co/kg may be spurious. It could also be argued that the results of the 49.8-mg WC-C0/kg dosage are spurious, and that the threshold for this effect is 16.6 mg WC-Co/kg; however, if that is the case, then lower dosages would be without effect and could not be considered mutagenic. Either way, it is important to note that there were dosages of WC-Co that did not result in a statistically significant increase of multinucleated AT-II cells, something that was not discussed or recognized by the DFG.

Additionally, it is possible that the statistically significant increase in micronucleated AT-II cells was in part, or in whole, the result of inflammatory cells brought about by pulmonary toxicity. In this study, intratracheal instillation of the primary dosage investigated – 16.6 mg WC-Co/kg – resulted in moderate, rather than mild (as was indicated in the abstract) pulmonary toxicity. This finding is of concern due to the fact that secondary genotoxicity can occur as a result of excessive and persistent formation of active oxygen species by inflammatory cells. This fact was recognized by the investigators themselves, who observed that "given the presence of intruding inflammatory cells, one cannot exclude that the mutagenic events detected in the AT-II cells are, besides being due to genotoxicity of the WC-Co particles, the result of inflammation (secondary genotoxicity)." Moreover, the investigators noted that, at 12 hours post-instillation of WC-Co, a substantial increase in inflammatory cells was observed, and "their presence in the alveoli before and during the

proliferation of the AT-II cells could contribute to the formation of the observed micronuclei." Another key difference reported by De Boeck et al. (2003a) was that, at later time points, the number of micronucleated type II pneumocytes after WC-Co treatment (16.6 mg WC-Co/kg) decreased, while this frequency remained higher in the positive control (bleomycin) rats. The authors indicated that this difference might be related to the fact that *cobalt was rapidly removed from the lungs* and that, at that time point, a lower frequency of inflammatory cells was present in the alveoli, both factors contributing to reduce the burst of active oxygen species released in the alveoli. Likewise, the observation of a statistically significant increase in DNA migration in AT-II cells at 12 hours after instillation of WC-Co (and the return to baseline afterward) could be related to the presence of *inflammatory cells exerting oxidative stress.* All in all, De Boeck et al. (2003a) concluded that further studies should be done to better understand the specific involvement of inflammatory cells in the formation of multinucleated AT-II cells. Therefore, it is possible that the statistically significant increase in micronucleated AT-II cells observed at the dosage of 16.6 mg WC-Co/kg was not due to a direct genotoxic effect of WC-Co on these cells but, rather, was due to secondary genotoxicity resulting from reactive oxygen species being generated from inflammatory cells as a result of pulmonary toxicity.

In addition to the above, there are other aspects of the study by De Boeck et al. (2003a) that may limit its usefulness in classifying hardmetal dust as category 1 carcinogens. First, the WC-Co used in this study consisted of 6.3% cobalt, 84% tungsten, and 5.4% carbon. Whether or not similar results would be observed with other WC-Co mixtures was not investigated, thereby limiting the results of this study to this particular mixture. Second, the median particle size of the WC-Co mixture used was 2 micrometers (μm). Thus, the vast majority of this mixture, if not all, was clearly respirable. Whether or not such a condition would occur in an occupational setting needs to be elucidated, but, if not, the results obtained in this study may not be relevant to humans who would likely be exposed to a range of particle sizes. Finally, the route of exposure used in this study – intratracheal instillation followed by a bolus dose of air – is a route by which humans would not be exposed. It is possible that this route of exposure overwhelmed the animals' respiratory defense mechanisms, thereby leading to toxicity. Whether or not such an effect would be observed in animals exposed via inhalation must be elucidated.

In summary, the DFG report does not provide a complete analysis of the study by De Boeck et al. (2003a). In fact, the data presented in the DFG report are incomplete, at best. While the DFG report presents the conclusion of the investigators, a more rigorous analysis of the data suggests that their conclusions may be in error. Indeed, a later review of the data by the investigators themselves cast doubt on the finding of a statistically significant

increase of DNA migration, as measured in the comet assay, at 12 hours following treatment with 16.6 mg WC-Co/kg. Moreover, the investigators' finding that only the 16.6 mg WC-Co/kg dosage resulted in a significant increase in micronucleated AT-II cells, while other dosages (even a high dosage) did not was not reported in the DFG report. Nor was the possibility that some, or all, of the reported genotoxic effects observed in this study could be due to inflammatory cells resulting from pulmonary toxicity, as noted by De Boeck et al. (2003a) themselves. All in all, the *in vivo* data relied upon by the DFG to support its classification of WC-Co as a category 1 carcinogen is incomplete, and the DFG's reliance on these data appears unwarranted.

Moreover, the DFG's reliance on the study by De Boeck et al. (2003a) ignores a study by De Boeck et al. (2000) in which no genotoxic (measured using the alkaline comet assay and the micronucleus test) effects were observed in peripheral blood lymphocytes of workers employed at cobalt refineries and hardmetal plants who were currently exposed to the threshold limit value/time weight average for cobalt-containing dust (20 micrograms of cobalt per cubic meter of air [20 µg Co/m^3]). While the DFG noted that "the authors [De Boeck et al., 2000] note that these results are not representative for the evaluation of the genotoxic effects of hardmetal dust for persons with higher exposures," the lack of evidence of genotoxicity in these workers clearly demonstrates that not all exposures, and hence not all doses, of WC-Co are associated with genotoxicity. Because the DFG seems to imply that a threshold exists below which WC-Co powders do not cause genotoxicity, animals studies must be performed using realistic exposure levels and applicable routes of exposure to have any meaningful utility.

Therefore, in light of the above, it would appear that the DFG may have placed too much reliance on the study by De Boeck et al. (2003a) in support of its classification of hardmetal dust as category 1 carcinogens. Therefore, the data by De Boeck et al. (2003a) should not be relied upon in categorizing hardmetal dust as category 1 carcinogens.

Overall, there is insufficient evidence upon which to make a judgment as to whether colbalt-tungsten carbide mixtures are genotoxic to occupationally exposed humans.

The limited experimental evidence obtained from studies on the impact of oxygen radicals on cellular DNA may provide some initial insight into a possible mechanism of chemical carcinogenesis. These data, however, are too limited, conflicting, and insufficient to support the hypothesis that the mixture of cobalt and tungsten carbide is capable of transforming normal human pulmonary cells into fatal, highly malignant derivatives. This purported

mechanism has not been conclusively confirmed and remains only a hypothesis. As such, the criterion of biological plausibility has not been fulfilled.

Elimination of Confounders

Smoking is perhaps the most significant "confounder" for lung cancer. Hogstedt and Alexandersson attempted to minimize smoking contribution by pointing out that "the percentage of current and former smokers does not appear to exceed the percentages among men nationally during the same period, even if there are no direct comparison numbers." Because there is "no direct comparison" information, the limited analysis does not address the potential for mischaracterization of smoking history on an individual basis. This is especially important given the low number of deaths – mischaracterizing one death from lung cancer that may have been because of smoking would alter significantly the SMR and the findings of the report. Unlike other epidemiological studies dealing with exposure to hardmetal, there is nothing in the report that details an attempt to determine whether an individual whose cause of death was lung cancer had a history of smoking. This was not described in this study; only the percentage of smokers and nonsmokers in the total cohort were provided. Thus, the issue of individual confounding could not be addressed in assessing the significance of the finding reported in this investigation. In fact, the authors acknowledged "the lack of good data on alcohol and tobacco usage."

Likewise, the authors of the Moulin study inadequately accounted for smoking history. Perhaps as evidence of this flaw, the OR for lung cancer associated with smoking was only 3.38. This is significantly lower than the OR typically reported for deaths associated with smoking (Hill, 1965; IARC, 1986). The authors acknowledged that this low risk associated with smoking may be due to misclassification. Other inadequacies in addressing smoking as a confounding variable include:

- None of the 61 cancer cases were actually interviewed for smoking histories (e.g., lack of direct information in medical or work histories).
- No medical records were reviewed for independent confirmation of self-reported or proxy-reported smoking histories.
- 70.5% (43 cancer cases) of the smoking history was obtained from colleagues.
- 11.5% (7 cancer cases) of the smoking history was obtained from relatives.
- 18% (11 cancer cases) had **no** information on smoking.

Without accounting for perhaps the most important confounder for lung cancer, these authors cannot make any determination regarding the cause-and-effect relationship, or even the association, between hardmetal exposure and lung cancer.

In addition to the problems associated with accurately characterizing the most important confounding variable for lung cancer, an unacceptably high number of workers were lost to follow-up. More than 15% of the exposed population was not accounted for in this study (1,131 workers). Inclusion of these workers undoubtedly would have changed the reported findings of the study – they might have increased or decreased the SMR. In any event, this rendered the study uninterpretable, and the authors should not have completed the study until these workers were found.

Inadequate evaluation of cigarette smoking as a confounder for lung cancer also plagued the Wild et al. report. The following paragraph taken from the published report highlights the uncertainty in the assessment of the possible contribution to lung cancer from smoking:

> *Exposure to smoking was abstracted from the records of the occupational health department; however, the information was sketchy until 1978, when current smoking or non-smoking was recorded but no mention was made of past smoking. Therefore, this information was reassessed by a volunteer group of former workers.*

This indicates that quantitative information on smoking histories was unavailable until 1978, which is almost 30 years after the initiation date for exposure considered in the study – January 1950. Even after the improvement in record keeping, the use of "a volunteer group of former workers" to obtain historical information was likely inadequate, although the authors did not provide any critical analysis of effectiveness of this approach.

Likewise, Lasfargues et al. relied on plant medical records and interviews of still active workers, and not specific information from cancer cases, to categorize smoking habits into individuals who never smoked, ex-smokers, and current smokers. However, this may have been the best of the studies in extrapolating smoking history because the profile of the proportion of smokers and former smokers in the hardmetal worker cohort closely matched the proportion in the national sample.

A careful review of the epidemiological studies clearly indicates that none of the investigation teams adequately addressed the most significant confounding variable in studies of lung cancer in human populations – cigarette smoking. Because the smoking histories were not quantitatively defined in the populations of workers, there is

no way of knowing the contribution of this known cause of lung cancer on the observed results. This is especially troubling given the low number of deaths contained in the studies (especially in Wild et al.) and the relatively low SMRs reported for lung cancer. Only a few cases misclassified as to their smoking status would have a dramatic impact on the interpretation of a casual relationship between hardmetal exposure and lung cancer.

Another potential confounding variable that was specifically identified in the Moulin et al. and Wild et al. investigations was co-exposure to chemicals identified by the IARC has causing cancer in humans. In fact, Wild and co-workers quantitatively evaluated this confounding variable and identified a statistically significant increase in lung cancer associated with exposure to "any IARC carcinogen." More than half of the 46 lung cancer deaths among workers exposed to other compounds in addition to hardmetal were in this category. The impact of this co-exposure on the interpretation of a causal association between hardmetal exposure and lung cancer was not explained by these authors.

Thus, given the potential misclassification of smoking status, and the acknowledged exposure to other potential cancer causing compounds, none of these studies satisfied the confounding criterion.

Coherence of Evidence

As previously stated, the limited and conflicting data available from *in vitro* and *in vivo* studies do not provide a scientifically grounded, biologically plausible mechanism supporting a cause-and-effect interpretation of the purported association between occupational exposure to colbalt-tungsten carbide mixtures and lung cancer. Additional evidence contained in the two major epidemiological studies also leads to challenges of the consistency of the body of scientific evidence.

Generation of reactive oxygen species leads to cell damage and structural changes in the lung. The types of pulmonary changes expected from the biochemical reactions initiated by oxygen radicals would include fibrotic changes (Lison and Lauwerys, 1992). This sort of insult has been observed in Hardmetal Disease. Thus, one would expect evidence in the epidemiological studies if this biochemical mechanism were possibly involved in the etiology of cancer in hardmetal workers.

However, Moulin et al. stated "this study failed to confirm the known pulmonary toxicity of hardmetals." The absence of deaths from fibrosis (including Hardmetal Disease) and pneumoconiosis suggests that high exposure

sufficient to cause frank lung toxicity was not present in these workers. The following are mortality incidence from the Moulin et al. cohort:

For Pneumoconiosis:

Men - 3 observed versus 2.25 expected
Women – 0 observed

For Fibrosis
Men – 0 observed versus 0.62 expected
Women – 0 observed.

Similar findings were reported by Wild and co-workers:

For Pneumoconiosis:
Men - 1 observed versus 0.55 expected
Women – 0 observed

For Fibrosis
Men – 0 observed versus 0.27 expected
Women – 0 observed.

These data do not identify the *incidence* of either fibrosis or pneumoconiosis within the populations and, therefore, do not provide a complete picture of lung injury in either the exposed or unexposed populations. However, one would expect that, if the incidence of either disease was significantly elevated, it would be reflected in increased mortality from the injury. This was not observed. This additional information, used in concert with the epidemiological and experimental data, fails to lay out a logical and consistent argument supporting a cause-and-effect relationship. Thus, the body of scientific information fails to satisfy the criterion of coherence.

6. Summary

In October 2003, the International Agency for Research on Cancer (IARC, 2003) released a monograph providing the new cancer classification for hardmetal. The Agency concluded that several epidemiological studies conducted in France provided evidence of an increased lung cancer risk related to exposure to hardmetal dust containing cobalt and tungsten carbide. As a result of its analysis, IARC characterized cobalt metal with tungsten carbide as *probably carcinogenic to humans* (Group 2A) on the basis of *limited evidence* in humans for increased risk of lung cancer. Similarly, the National Toxicology Program (NTP) recently requested public comment on its review of cobalt/tungsten carbide hardmetal manufacturing for possible listing as a "known human carcinogens and reasonably anticipated human carcinogens" based on the available epidemiological evidence. Consistent with the activities of these scientific organizations, the DFG (German Research Foundation) recently placed hardmetal dust in "category 1 carcinogens" since they "cause lung cancer in humans."

In order for a compound to be categorized as a known or probable human carcinogen, the available data must demonstrate a cause-and-effect relationship. There is a broadly accepted, scientifically objective methodology available to evaluate these data and establish the causal relationship between chemical exposure and cancer. This methodology, referred to as a causation analysis, was used to evaluate the available epidemiological studies on hardmetal exposure and lung cancer, the reports by Hogstedt and Alexandersson (1990), Lasfargues et al. (1994), Moulin et al. (1998), and Wild et al. (2000).

These studies were plagued by study design weaknesses (e.g., low number of deaths in the cohorts); uncertainties, particularly in estimating exposure (and therefore dose); and an inability to address important confounding variables (especially for cigarette smoking). Thus, the weak associations reported by these investigators cannot be used to support the determination that hardmetal dust is a known human carcinogen. The data are simply too weak, and, as illustrated by the causation analysis provided in Section 5, an objective evaluation of these reports demonstrates the limitations of the available epidemiological data in establishing a cause-and-effect relationship. Without establishing the cause-and-effect relationship, hardmetal dust cannot be characterized as either a "known" or "probable" human carcinogen.

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Annex 2

Lison, D., Arras, M., Fubini, B., and Prandi, L. 2001.

TOXI-report

In vitro tests on cobalt, cobalt compounds and mixtures with tungsten carbide particles

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KENNAMETAL c/o K. Goerting

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6

Background.

Previous experimental observations (Lison et al. 1995) have suggested that the lung toxicity of hard metals (lung alveolitis and cancer) could be mediated by the production of activated oxygen species (AOS) which arises from the reduction of molecular oxygen in biological media by metallic cobalt (Co), a process that is catalysed at the surface of tungsten carbide particles (WC). This hypothesis is supported by several other experimental observations.
Kenametal has developed a new material (Co_3W) that is proposed to replace Co in the manufacture of hard metals.

Objective.

The objective of the test is to examine whether WCo_3 alone or mixed with WC particles is susceptible to cause the same toxic concerns as classical hard metals. Two tests that proved useful with the hard metal particles (WC-Co) and are believed predictive of a toxic potential are used :

- a cell free assay to measure the capacity of hydrogen abstraction which indirectly assesses the amount of AOS formed by the particles in a suitable electrolyte.
- a macrophage cytotoxicity assay to evaluate the capacity of the particles to damage biological materials.

Materials.

The following materials were provided by Kennametal (Mr K. Goerting) in April 2001:

- cobalt metal (Co), 50 g
- Co_3W, 50 g
- tungsten carbide (WC), 50 g, labelled 1µ
- a WC:Co mixture (90 g:10 g)
- a WC:Co_3W mixture (80 g:20 g)

No indication of the mode of production of these powders was provided and their characteristics (shape, size, specific surface area, microphotography) are not further detailed.
In the course of the testing, a WC-Co mixture (94:6) reconstituted in the laboratory from WC and Co or a commercial WC-Co powder[1] (94:6) were included in the H-abstraction and cytotoxicity assays, respectively.

The methodological procedures for both assays was described in details in Lison et al. 1995[1]

[1] Lison D, Carbonnelle Ph, Mollo L, Lauwerys R and Fubini B (1995) Physico-chemical mechanism of the interaction between cobalt metal and carbide particles to generate toxic activated oxygen species. Chem Res Toxicol 8:600-606.

Results

H ABSTRACTION ASSAY.

Experimental: 1000 μL of a solution 0.15 M of DMPO was added to a suspension of 45 mg of sample (no attempt was made to normalise for cobalt content) : the solution was stirred at room temperature and then 1 mL of a 1.0 M solution of sodium formate in sodium phosphate buffer was added. The EPR spectra were recorded on aliquots of 50 μL of the suspension at 10, 30 and 60 minutes. An Adani EPS100x EPR spectrometer operating at X band (9-9.5 GHz) was employed. The yield in free radicals is proportional to the intensity of the signal, which was measured by double integration.

Results: Table 1 compares the mean intensity values obtained for all the samples submitted to the test after 30' in two independent assays.

Table 1

Sample	Arbitrary units
WC	0
Co	0
WC-Co_3W	51.8
WC-Co 90:10	31.2
Co_3W	4
WC-Co 94:6	*65.2*

The kinetics of release of $CO_2^{\cdot-}$ radicals for the different samples are reported for all the samples in Fig 1.

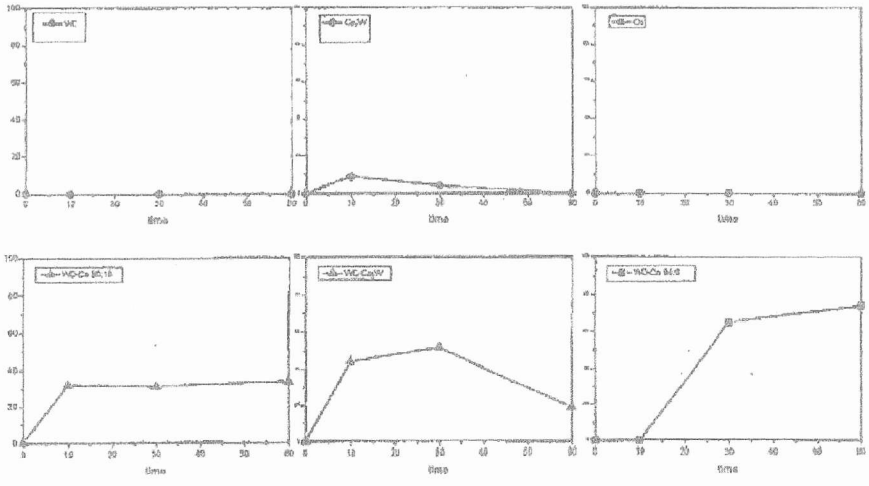


Figure 1

MACROPHAGE CYTOTOXICITY ASSAY

Experimental : The powders were sterilised during 2 h at 200°C before use.
The cytotoxicity of the different powders was assessed by measuring the release of LDH after 18 h culture in the presence of increasing doses.
The cells were obtained by peritoneal lavage of mice injected intraperitoneally 3 days with 1 ml of casein hydrolysate (6%). The peritoneal macrophages (0.8×10^6 cells per well) were purified by adherence during 3 h in DMEM + 10 FCS and the cultures were rinsed with PBS before addition of the powders. All stock suspension were prepared immediately before use in milliQ water.
After 18 h of incubation with the appropriate dose of powders suspended in DMEM without FCS (0.1 lactalbumin hydrolysate), the culture media were harvested, centrifuged to eliminate cells debris and measured for their LDH activity. Controls were incubated in culture medium alone. The LDH release was expressed in % of total cellular LDH content determined in parallel after complete lysis of the cells in 0.1 % Triton X100. Each dose was tested in quadruplicate and results are presented as mean ± SD.
A first experiment was performed with the following doses :

- controls
- Co : 5, 10 and 20 µg/well
- WC : 45, 90 and 180 µg/wel
- WC-Co (90:10) : 50, 100 and 200 µg/well
- Co_3W : 10, 20 and 40 µg/well
- WC-Co_3W : 50, 100 and 200 µg/well.

A second experiment was conducted with the following powders and doses :

- controls
- Co : 5, 10 and 20 µg/well
- WC : 45, 90 and 180 µg/wel
- WC-Co (90:10).: 50, 100 and 200 µg/well
- WC-Co (94:6) : 100, 200 and 400 µg/well
- Co_3W : 10, 20 and 40 µg/well
- WC-Co_3W : 50, 100 and 200 µg/well.

Results : The results of the first and second experiments are presented in Figures 2 and 3, respectively. The dose is expressed in Co or Co_3W equivalents.

Figure 2

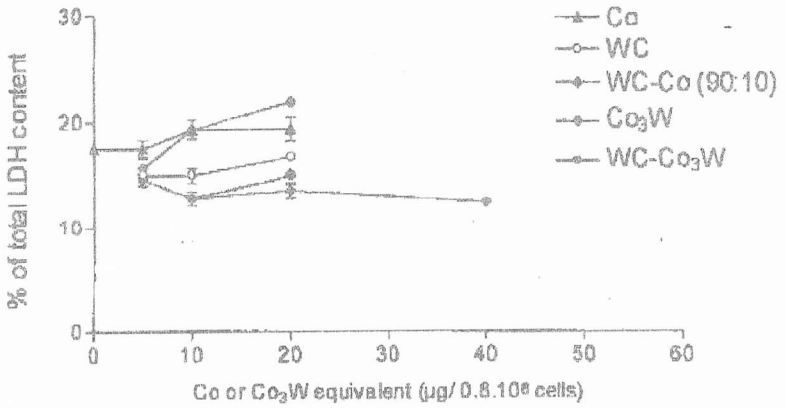
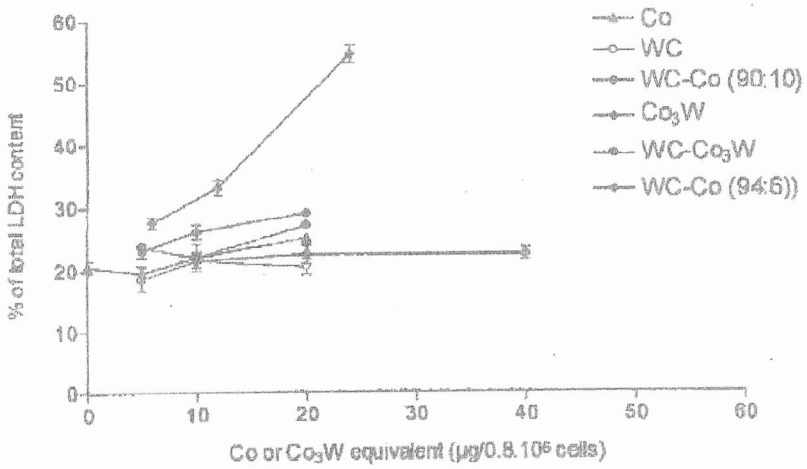


Figure 3



Intepretation : In the first experiment, none of the material tested did elicit cytotoxicity. Since this result was unexpected, in particular for WC-Co (90:10) which was included as a positive control in the test, a second experiment was carried out with another WC-Co (94:6) which proved cytotoxic in previous experiments. The results obtained in the first experiment were confirmed but WC-Co (94:6) exerted clear cytotoxicity.

Conclusions.

Co_3W did not produce AOS in the H abstraction assay. All mixtures of WC with Co or Co_3W were found active in the same assay. The activity of WC, Co and WC-Co (94:6) in this assay are consistent with previous observations and the results can be considered as valid.

In the cytotoxicity assay, none of the powders provided by Kennametal did produce cytotoxicity. The reason for the absence of cytotoxicity of the WC-Co (90:10) sample which was introduced as a positive control remains unclear. The clear activity of WC-Co (94:6) in the same assay validates the sensitivity of the test system. No conclusion on the cytotoxic potential of the test samples can be drawn.

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Annex 3

Pardus, M., Lemus-Olalde, R. and Hepler, D. 2009 (accepted for publication).

Tungsten human toxicity: A compendium of current research on tungsten and tungsten compounds

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ABSTRACT

Over the past several years, a number of tungsten toxicology studies have been conducted by various investigators, primarily by researchers associated with the U.S. military. Only a few of these studies have been published in the peer-reviewed literature; however, the results have been presented at numerous scientific meetings and produced as part of formal scientific reports (need citations). Nonetheless, a picture is starting to emerge from these tests that suggest that tungsten exhibits little relative human health toxicity. This paper provides an overview of those studies along with their conclusions. The intent of this paper is not to produce a detailed description or analysis of these studies, but to provide highlights of the current tungsten research activities that may be of interest to the scientific community but that have not been addressed or compiled in other venues.

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KEYWORDS

Tungsten, sodium tungstate, health effects, toxicology, U.S. military, Department of Defense

INTRODUCTION

Although tungsten metal is, and has been, widely used for decades for a number of industrial uses, there is little in the available toxicological literature to indicate that tungsten is associated with toxicological endpoints identified for other heavy metals (e.g., neurological effects in some lead exposed individuals, cancer in some lead, arsenic and chromium exposed individuals).

Concerns, however, have been raised about the toxicity of tungsten metal. These concerns appear to stem from the identification of a cancer cluster – specifically acute lymphoblastic leukemia – among a group of children who reside in Fallon, Nevada (an area will high levels of naturally occurring tungsten) and growing interest on the part of the U.S. military in tungsten-based munitions and ordnance.

Extensive investigations by the Centers for Disease Control (CDC) and the Nevada State Health Division found no evidence upon which to implicate tungsten as the cause of the Fallon cancer cluster (Rubin et al. 2007). However, the Agency for Toxic Substances and Disease Registry (ATSDR)

acknowledged that there was only limited data regarding human health effects of tungsten (ATSDR 2005). Consequently, the CDC nominated tungsten for evaluation by the National Toxicology Program (NTP) (Rubin et al. 2007).

Over the past several years, a number of tungsten toxicology studies have been conducted by various investigators, primarily by researchers associated with the U.S. military. Only a few of these studies have been published in the peer-reviewed literature; however, the results have been presented at numerous scientific meetings and produced as part of formal scientific reports (need citations). Nonetheless, a picture is starting to emerge from these tests that suggest that tungsten exhibits little relative human health toxicity.

The term “tungsten” has been loosely applied in the technical literature to a variety of compounds, alloys and composite materials. Tungsten heavy alloys are generally based on systems based on W-Ni-Fe, W-Ni-Cu (with or without iron), and in some instances (W-Ni-Co) (Lassner and Schubert 1999; Kalinich, et al. 2005). Cemented tungsten carbide (also referred to as hardmetal) consists of a group of refractory composites in which tungsten carbide particles are bound together by a matrix metal (usually cobalt) along with other auxiliary metals and/or metal carbides to achieve desired physical and wear properties (Lassner and Schubert 1999).

To avoid confusion, this paper focused on research activities related to tungsten metal and/or individual tungsten compounds. Much of the available research on tungsten metal and related compounds has used sodium tungstate due to its solubility in water and ease of dosing test animals. As the most soluble, and biologically available form of tungsten, selection of sodium tungstate for toxicity testing is appropriate.

SUMMARY OF RESULTS FROM RECENT TUNGSTEN PRESENTATIONS AND STUDIES

As noted previously, there are a number of investigations that have been conducted or are underway to address tungsten toxicity data gaps identified by the ATSDR and by the military. A number of these studies on tungsten substances are in the process of being published and have been presented at various technical conferences particularly over the past year. Many of these studies have been conducted by military (Army, Navy, and Air Force) or governmental (NTP, NIOSH) researchers.

This section provides an overview of those studies along with their conclusions. The intent of this paper is not to produce a detailed description or analysis of these studies, but to provide highlights of the current tungsten research activities that may be of interest to the scientific community but that have not been addressed or compiled in other venues.

Oral Subchronic Toxicity Studies

As a follow-up to earlier work (CHPPM 2006), a 90-day oral toxicity study was conducted on male and female rats which were exposed by gastric intubation to 0, 10, 75, 125 and 200 mg/kg/day. Results showed findings in the kidney of males and female rats at the two highest doses, in the stomach of males (at all doses) and females (with the exception of the lowest dose), and in the male reproductive system only at the highest dose. As the histological findings within the stomach are not considered adverse, the No Observable Adverse Effect Level (NOAEL) is 75 mg/kg/day based on renal changes (CHPPM 2008). The authors indicated that the adverse effects observed in this study are similar to effects produced by other metals (CHPPM 2008).

Toxicokinetic Studies

The disposition of tungsten (administered as sodium tungstate in water) in plasma, liver, kidneys, uterus, femur, and intestine of rats and mice was characterized after exposures by oral gavage to 1, 10, or 100 mg/kg. Tissue and plasma was collected and analyzed by inductively coupled plasma mass spectrometry at 1, 2, 4, or 24 h after dose administration. Tungsten was observed in plasma and all tissues and concentrations in plasma and most tissues peaked at 4 h. In mice, concentrations in plasma and most tissues peaked at 1 h. Although the amount of tungsten in each matrix decreased

significantly by 24 h, there was tungsten remaining in several tissues, especially at the higher doses (McDonald et al. 2007).

Two separate toxicokinetics studies exposed rats via nose-only inhalation with one nostril occluded to radioactive sodium tungstate for 90 minutes to a concentration of approximately 250 mg $^{188}W/m^3$. In one of the studies the left and right sides of the nose and brain were sampled at 0, 2, 3, 7 and 21 days post-exposure. Results demonstrated that tungsten will not be appreciably transported via the olfactory pathway to the brain (Olabisi et al. 2008a).

The second study sampled tissues at 0, 1, 3, 7, and 21 days post-exposure and analyzed using gamma-spectrometry. Relatively rapid tungsten elimination (apparent half life of elimination was less than 1 day) was observed from most tissue compartments. However, tissues with slower terminal elimination rates included femur, lung, and spleen (Wagner et al. 2008).

Reproductive / Developmental Studies

The toxic effects of the oral administration of sodium tungstate for 70-days was determined in parental rats (P_0) and in the reproductive, systemic and gestational effects of sodium tungstate on the P_0, as well as early growth and developmental effects in the offspring. In addition, behavioral effects were measured in the offspring of the low and high dosed groups using: 1) auditory reflexes by acoustic startle, 2) pre-impulse inhibition responses, 3) maternal

retrieval, 4) water maze test (learning memory), and 5) spontaneous gross locomotor activity. Rats were exposed to 5 (low), 62.5 (mid) and 125 mg/Kg-day (high).

Differences from the control were observed at the mid and high doses for both tungsten distribution in organs and organ weights. Tungsten accumulated in parental and pups; showing a systemic distribution in the gastrointestinal tract, femur, kidney, lung, and thymus. At the high dose degeneration of heart cell and necrosis was observed in parental males. Neuro-developmental effects (righting reflex) were reported for male pup at low and high concentrations. A review by others suggests that the righting reflex was not an adverse affect due to inconsistencies in the reported data, e.g. neither males nor females exhibited a dose-related statistically significant change in the righting reflex (Schell, 2008)**.** Nonetheless, the study's authors concluded that along with the neurodevelopmental effects at low doses, oral exposure to high levels of sodium tungstate caused signs of systemic toxicity (e.g., mild myocardial degeneration) in rats (McInturf et al. 2007).

Immunotoxicity Studies

Two immunotoxicity studies on sodium tungstate were identified. One of the studies measured spleen and blood signaling proteins (cytokines) from orally exposed rats. Results showed no statistically significant differences between

parental and offspring rats, or between treated and control groups (Prues et al. 2008).

The second immunotoxicity study using *in vitro* assays provide evidence that tungsten concentrations of 1 to 50 millimoles (equivalent to 184 to 9,192 mg/L) produced measurable effects on cells both under stimulated and unchallenged conditions by influencing immune signal pathways, cell cycle progression, cell migration, and cell death (Carson et al. 2008). However, it is unclear whether the *in vitro* assay, and the concentrations tested, has relevance to disease attributable to tungsten at environmentally relevant levels.

Genotoxicity Studies

Chromosomal damage determinations in immune blood cells of parental rats exposed to 62.5 mg/kg-day of sodium tungstate for 70-days revealed frequent deletions, but with no clear difference observed between treated and control rats (Prues et al. 2008).

Sodium tungstate or tungsten powder did not produce mutagenic effects at any dose up to 5,000 μg/plate in *Salmonella typhimurium* or *Escherichia coli* tester strains with and without a metabolic activation (S-9). Neither sodium tungstate nor tungsten powder increased mutation frequency at levels up to 350 and 5,000 μg/mL, respectively, in mouse lymphoma cells in the presence or absence of S-9. The effect of sodium tungstate or tungsten powder on *in-*

vitro chromosomal aberrations in Chinese Hamster Ovary (CHO) cells showed no effect for inducing structural chromosomal aberrations up to 3,500 and 500 μg/mL, respectively, with and without S-9. Sodium tungstate was not clastogenic/aneugenic to the mouse bone marrow when tested in the *in-vivo* mouse micronucleus assay up to 750 mg/kg. The authors concluded that sodium tungstate and tungsten powder were not genotoxicants in all of the above tests conducted (Reddy et al. 2007).

Carcinogenic Studies

Rats were surgically implanted with 4 (low dose) or 20 (high dose) pellets of tungsten. A negative control group had 20 pellets of tantalum and a sham surgery control group were also included in the study design. Effects were assessed 1, 3, 6, 12, and 22-months post-implantation.

No rats implanted with tungsten pellets developed clear adverse effects as a result of the pellet implantation, nor were any tumors detected in these animals. No significant changes in body weight, clinical chemistry, hematology or urinalysis parameters were seen in rats implanted with pellets of tantalum or tungsten.

At 1 and 3-month post-implantations tungsten pellets caused mild to moderate tissue damage compared to the negative control pellet group. However, by

month 12 the inflammation had resolved, and no treatment related changes were noted (Roszell et al. 2008).

CONCLUSIONS

An assessment of the available toxicological literature and the results of current research activities presented herein indicate that tungsten is associated with mild adverse toxicological effects. A preliminary evaluation of risk-based concentrations of tungsten in soil and drinking water, suggests that tungsten is generally non-toxic and is similar to other relatively innocuous metals such as tin and zinc in this regard (Schell, et al. 2007a and 2007b).

Despite decades of use of tungsten and individual tungsten compounds in various industries, there is little in the way of evidence that tungsten in the environment is of toxicological concern to humans, particularly to individuals with no occupational exposures. Some might suggest that this finding stems from a lack of investigation of the effects of tungsten in humans. However, an equally plausible explanation is that tungsten simply does not cause harm to humans under ordinary conditions of use, or from known environmental exposure.

Current tungsten research endeavors continue to fill data gaps previously identified by the ATSDR for tungsten (ATSDR 2005). However, these

ongoing tungsten studies need to enter the realm of peer-reviewed scientific literature to be of value to policy makers and regulatory agencies.

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